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- (71) Applicant: CENTOCOR, INC. [US/US]; 200 Great Valley Parkway, Malvern, PA 19355 (US).
- (72) Inventors: SNYDER, Linda; 1795 Honeysuckle Lane, Pottstown, PA 19465 (US). SCALLON, Bernard; 139 Hemlock Drive, Collegeville, PA 19426 (US). KNIGHT, David, M.; 2430 Whitehorse Road, Berwyn, PA 19312 (US). MCCARTHY, Stephen, G.; 701 Patrick Henry Circle, West Chester, PA 19382 (US). GOLETZ, Theresa, J.; 147 Pinecrest Drive, King of Prussia, PA 19406 (US). BRANIGAN, Patrick, J.; 223 Hansell Road, Lansdowne, PA 19050 (US).

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(54) Title: NUCLEIC ACID VACCINES USING TUMOR ANTIGEN ENCODING NUCLEIC ACIDS WITH CYTOKINE ADJUVANT ENCODING NUCLEIC ACID

(57) Abstract: Nucleic acid vaccines are provided that comprise at least one tumor antigen encoding nucleic acid and at least one cytokine adjuvant encoding nucleic acid for prophylaxis or treatment of tumors. The viral vaccines of the invention are optionally combined or additionally administered with a recombinant virus or DNA vaccine booster.

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NUCLEIC ACID VACCINES USING TUMOR ANTIGEN ENCODING NUCLEIC ACIDS WITH CYTOKINE ADJUVANT ENCODING NUCLEIC ACID

FIELD OF THE INVENTION

The present invention relates to nucleic acid vaccines comprising sequences that encode a tumor antigen as an immunogen and a cytokine as an adjuvant. The vaccines are suitable for the vaccination of mammals, including humans, in order to provide unexpectedly enhanced cellular and/or humoral immune responses to one or more tumor related pathologies. Additionally, the invention relates to methods for making and using such nucleic acid vaccines.

BACKGROUND OF THE INVENTION

Cancer is a serious disease that afflicts one in four people. In the last fifty years, there have been significant improvements in the early detection of cancer, as well as the development of a number of therapies to treat cancer. Therapies include surgery to remove primary tumors, and sublethal radiation and chemotherapy to treat disseminated disease. While these treatments have resulted in apparent cures for many patients, the treatments can be quite debilitating and are still often ineffective at preventing death from this disease. There is clearly a need for therapies that are less destructive, as well as for novel therapies that harness the body's natural defenses to fight cancer.

Cancer can be divided into two classifications, depending upon the cell type the tumor is derived from. For example, carcinomas are derived from epithelial cells, while sarcomas are derived from mesodermal tissues. Some epithelial tumors express on their surface a protein called mucin 1 (MUC1).

MUC1 is a transmembrane protein that is normally expressed in non-disease states on ductal epithelial cells, such as those in the intestinal mucosa exposed to the lumen of the small intestine. The most notable feature of MUC1 is its large extracellular domain, which is comprised of 30-100 tandem repeats of a 20 amino acid sequence. The tandem repeats confer a rigid structure to this portion of the protein, and the repeats are a substrate for heavy glycosylation. In addition, in normal cells MUC1 is only expressed on the ductal side of the cell. It is thought that MUC1 may provide a lubrication function to the duct, and it may also be involved in signal transduction. Because the protein is normally expressed on the ductal side of cells, it is rarely exposed to the outside of the organism, and is considered a "sequestered antigen", because in its native form MUC1 is not exposed to immune system surveillance.

In contrast, MUC1 expression is different in epithelial tumors. The protein becomes overexpressed and is present all over the surface of the cell, and it is relatively deglycosylated as compared to the normal form expressed in ductal epithelial cells. Thus, the distribution and pattern of expression is very different in normal and neoplastic tissues, and the deglycosylated, aberrant protein exposes novel epitopes to the immune system. Because the pattern of expression is different from normal, it is possible that the immune system can now recognize the tumor-associated MUC1 as foreign and attempt to destroy the cells expressing this protein. Indeed, the immune system does appear to act in this way in some cancer patients. It has been shown that patients with ovarian, breast or pancreatic cancer possess weak antibody and cytotoxic T lymphocyte (CTL) responses to MUC1, indicating that their immune systems do indeed recognize a difference in the tumor-associated MUC1. However, the immune responses are clearly not strong enough to eliminate tumor cells.

These observations have led some investigators to develop therapeutic strategies designed to induce or strengthen the natural immune response. For example, several groups have attempted to use MUC1 peptides to prime a cellular response in patients. This relies on the concept that cells could process the peptide and present it in the context of Class I molecules to the immune system, to cause a Th1 response to cells expressing the MUC1 protein. There are several disadvantages to known approaches. First, peptides have short half-lives, requiring administration of large amounts of the peptide. Second, each person expresses several Class I molecules and a given peptide binds to only one molecule, which will be held by a minority of the patient population. Third, the immunity generated by such approaches may not be relevant to treating such cancers; it has been noted that anti-peptide immunity can be generated by peptide immunization, which does not always lead to anti-protein immunity.

The identification of tumor-specific antigens has supported the concept that immunologic strategies could be designed to specifically target tumor cells in cancer patients. Immunologic recognition of tumor antigens has been subsequently documented in patients with malignancy. However, these responses are muted and are ineffective in eradicating disease. The development of immune tolerance towards malignant cells is due, in part, to the inability of tumor cells to effectively present antigens to the immune system. Therefore, T cells with the capability of recognizing these antigens fail to become activated. A major focus of cancer immunotherapy has been the attempt to introduce tumor antigens into the cancer bearing host such that they may be recognized more effectively and that meaningful antitumor responses can be generated. In this way, native immunity directed against antigens selective for or over-expressed in malignant cells may be amplified and result in tumor rejection. Approaches to induce tumor-specific immunity have included vaccination with tumor cell extracts, irradiated cells, tumor-specific peptides with

and without adjuvant, and dendritic cells (DC) pulsed with tumor peptides/proteins, or manipulated to express tumor-specific genes.

DNA immunization has been used as a method to generate immune responses *in vivo*, and has been recognized as an effective way to generate cytotoxic T cells directed against an encoded antigen. Vaccination with tumor-specific naked DNA results in the expression of tumor antigens by the inoculated muscle cells. Professional antigen presenting cells, in particular DC, recruited to the site of injection, internalize and subsequently present the tumor-specific antigens at sites of T-cell traffic.

Breast cancer is a common malignancy second only to lung cancer among cancer deaths in women. In 2000, it was estimated that 182,800 new cases were diagnosed and 41,200 deaths resulted from breast cancer in the United States (US). Standard-dose combination chemotherapy can yield high response rates in previously untreated patients with metastatic disease, but complete responses are rare. Despite initial chemosensitivity, median disease response duration is less than 1 year due to the emergence of chemoresistant disease. The median survival for patients with metastatic disease has remained approximately 2 years for those treated with standard-dose chemotherapy. A majority of breast carcinomas express MUC1. As noted in the Investigator's Brochure, responses to recombinant vaccine constructs expressing MUC1 have been shown to induce immune responses in mice and chimpanzees. As such, immunotherapeutic strategies targeting the MUC1 antigen are a potentially promising approach for patients with metastatic breast cancer who otherwise lack effective treatment options.

Prostate cancer is the second leading cause of cancer-related death in men. Approximately 180,000 men will be diagnosed with prostate cancer each year, and 40,000 succumb to the disease each year. Prostate tumor cells have a low proliferation rate and do not respond to standard chemotherapies, which are most toxic to the most rapidly dividing cells in the body. Instead, prostate cancer can be treated surgically, with radiation therapy or hormonal therapy. Surgery and radiation therapy can lead to undesirable side effects, such as incontinence and impotence. The disease can often be successfully managed with hormonal therapy, which starves the cells for its required growth factors. However, eventually all tumors treated in this way become androgen-independent and there is no effective treatment beyond that point. There is clearly an unmet medical need to treat this disease more effectively, and with novel therapies.

One such approach that has considerable promise is active immunotherapy. Active immunotherapy would stimulate the patient's immune system to generate an anti-tumor response that could help hold the disease in check longer, or even rid the patient of metastatic disease. One

example of active immunotherapy include dendritic cell therapies, where the patient's professional antigen presenting cells are removed and pulsed with tumor antigen, transfected with tumor RNA/cDNA, or fused with tumor cells. The ex vivo-treated dendritic cells are then reinjected into the patient, and are expected to drive a prostate-tumor specific immune response. One disadvantage of such approaches is that they amount to designer therapy that would be very costly and require very specialized skills to administer. Such therapies are unlikely in their current form to be widely used.

A second active immunotherapy approach is peptide vaccination. In this approach, tumor-specific peptides or proteins are administered to the patient, with the hope of directly loading antigen-presenting cells in vivo. This approach is more likely to be usable in the clinic than the ex vivo approach described above, but consistent success has not yet been achieved with this strategy. Some problems include the fact that peptides are short-lived in vivo, and therefore require very large doses. In some clinical trials, peptide vaccination engenders anti-peptide immune responses that do not translate into responses against tumors expressing the whole protein from which the peptides were derived.

A third active immunotherapy approach that has much more promise to be widely used would be a cancer vaccine. Specifically, we believe that a DNA vaccination approach could be very effective in treating prostate cancer patients. In this treatment, the vaccine would be comprised of plasmids (or other DNA-containing agents) that encode antigen(s) specific to prostate cancer. The plasmids would be injected into the patient, and the prostate-specific antigens would then be expressed and presented to the immune system. The antigen-presentation process would engender a specific cellular and/or humoral response that could help to control the growth of the tumor or its metastases. From preclinical models there is reason to believe that such an approach could be effective. For example, vaccination of rhesus monkeys with DNA vaccines encoding PSA +/- cytokine adjuvants drives PSA-specific humoral responses and cellular proliferation. In two male monkeys vaccinated in this way, there was evidence of infiltrating cells within the prostate post vaccination, but not in a nonvaccinated control. In work in our labs, we have shown that vaccination with DNA encoding a different tumor associated antigen, MUC1, can lead to immune responses protective against tumor challenge with MUC1-expressing tumors. Thus, it may be possible to use DNA vaccines to break tolerance to self-antigens that happen to be strongly expressed by tumors, and mount a therapeutic immune response.

While vaccination with PSA with or without cytokine adjuvants may very well be effective as an immunotherapy, it is possible that this would not be enough to control tumor growth. It is entirely

possible that an effective immune response against PSA would eliminate PSA+ tumor cells but leave PSA- prostate tumor cells intact and able to grow unfettered. Therefore, it may be desirable to vaccinate with more than one tumor antigen. We propose that a DNA vaccine comprised of the PSA antigen with other antigens expressed highly in prostate cancer, such as KLK2 and/or MUC1, and perhaps with other adjuvant/costimulatory genes, would be a more effective approach than vaccination with a single antigen.

PSA or KLK3 is a member of a multigene family known as the human kallikrein gene family. There are 15 closely related genes in the family, all of which map to a 300kb region of human chromosome 19q13.3-q13.4. Kallikreins are secreted serine proteases. All are synthesized as preproenzymes; proenzymes arise after removal of the signal peptide, and the mature active protease arises after removal of a propeptide. The activity of a given kallikrein will be either trypsin-like or chymotrypsin-like, depending upon the nature of the active site. PSA or KLK3 is a 30 Kd serine protease with chymotrypsin-like activity, which is responsible for cleaving seminogelin I, seminogelin II and fibronectin in seminal fluid. PSA is most highly expressed in the prostate, but it is also expressed at lower levels in breast, salivary gland, and thyroid. Besides prostate cancer, PSA is expressed in some breast malignancies. PSA has become well known as a serum marker for prostate cancer; it is a very important diagnostic for this disease and increasing serum levels of PSA typically correlate well with the severity of the disease. Expression of PSA is not increased in prostate cancer cells versus normal prostate cells; instead as the disease breaches the normal cellular barriers, PSA leaks into the serum. It is unclear if PSA has a role in the etiology of prostate cancer; various reports have indicated that PSA could either enhance or inhibit tumorigenicity. Several CTL epitopes for PSA have been described for the HLA A2 and A3 haplotypes; identification of these epitopes support the possibility of generating therapeutic in vivo CTL by vaccination.

KLK2 is the member of the kallikrein family that most closely resembles PSA, with about 80% identity at the amino acid level. Like PSA, KLK2 is expressed highly in the prostate and in prostate cancer, with lower levels of expression in other tissues, such as breast, thyroid, and salivary gland. KLK2 has trypsin-like activity, and one of its activities is to cleave the proenzyme form of PSA to yield the mature enzyme. There is increasing recognition that KLK2 may be a good serum prognostic indicator to monitor the progress of prostate cancer patients, although it is likely to be a supportive diagnostic along with PSA.

Accordingly, there is a long-felt and pressing need to discover vaccines and methods that elicit an immune response that is sufficient to treat or prevent various tumor related human pathologies.

SUMMARY OF THE INVENTION

The present invention is intended to overcome one or more deficiencies of the related arts. In particular, nucleic acid vaccines of the present invention advantageously provide a more robust immune response. The strength of the present invention lies in its power to recruit one or more of B cell, helper T cell, and cytotoxic T cell components of the immune response for effective humoral and cellular immunity.

To provide more effective tumor or cancer vaccines, the present invention provides nucleic acid vaccines comprising a cancer-specific or tumor-specific antigen nucleic acid and an adjuvant nucleic acid. Also provided are methods of making and using such nucleic acid vaccines. In their use as a vaccine, the co-expression of tumor nucleic acid and the adjuvant nucleic acid in a tissue to which the vaccine of the present invention has been introduced induces a cellular or humoral immune response, or any component thereof, to the tumor protein or fragment thereof.

This invention uses nucleic acids (or fragments thereof) encoding such tumor antigens as, but not limited to, prostate specific antigen (PSA), KLK2, and/or mucin-1 (MUC1) as antigen components of a DNA vaccine for tumors, such as but not limited to, any PSA, KLK2 or MUC-1 associated tumor or cancer. The antigen genes will be of human origin, or mutated to enhance their immunogenicity. Examples of how the antigen genes could be rendered more immunogenic would include alteration or removal of signal sequences required for secretion, optimization of codons for improved translation, addition of ubiquitination signals for degradation, addition of subcellular compartment targeting sequences, addition of molecular chaperone sequences, and optimization of CTL epitopes. The antigen genes could be fused together to increase immunogenicity. The CTL/helper epitopes could be linked together, or inserted as part of another molecule, such as an immunoglobulin molecule.

Other genes may also be included in the vaccine, including cytokine adjuvant genes such as IL-18, IL-12 or GM-CSF, or genes for costimulatory molecules such as B7-1, which would help to drive the immune response.

The genes of the invention could be encoded by plasmids, viruses, bacteria or mammalian cells. The vaccination regimen could be comprised of any or all of these agents, such as a plasmid DNA

priming vaccination, followed by a viral vector boost. The latter approach appears to be effective in generating cellular responses important in controlling infectious diseases (28-32), and may be very useful in anti-cancer applications of this technology as well.

In the vaccines of the invention, the tumor encoding nucleic acid may be isolated from patients having a tumor related cancer, preferably from the cancerous tissue itself or from mRNA or cDNA encoding a cancer-related tumor protein or antigenic portion thereof.

The present inventors have discovered that nucleic acid vaccines of the present invention elicit unexpectedly enhanced immune responses by the expression and/or presentation of at least one tumor antigen encoding nucleic acid and at least one cytokine adjuvant encoding nucleic acid.

The present invention also provides at least one tumor/adjuvant nucleic acid encoding (or complementary to) at least one antigenic determinant encoding nucleic acid of at least one tumor protein and at least one adjuvant encoding nucleic acid of at least one portion of an IL-18 protein.

The present invention also provides a tumor/adjuvant vaccine composition comprising a tumor/adjuvant nucleic acid vaccine of the present invention, and a pharmaceutically acceptable carrier or diluent. The vaccine composition can further comprise an additional adjuvant and/or cytokine encoding sequence or further component of the composition which enhances a nucleic acid vaccine immune response to at least one cancer associated tumor protein in a mammal administered the vaccine composition. A nucleic acid vaccine of the present invention is capable of inducing an immune response inclusive of at least one of a humoral immune response (e.g., antibodies) and a cellular immune response (e.g., activation of B cells, helper T cells, and cytotoxic T cells (CTLs)), with a cellular immune response preferred.

The present invention also provides a method for eliciting an immune response to a cancer associated tumor protein in a mammal which is prophylactic for a cancer associated tumor protein, the method comprising administering to a mammal a vaccine composition comprising a nucleic acid vaccine of the present invention, which is protective for the mammal against a clinical MCU-1-related pathology.

The present invention also provides a method for eliciting an immune response to a cancer associated tumor protein in a mammal for therapy of a tumor-associated pathology, such as but

not limited to a tumor or cancer. The method comprises administering to a mammal a composition comprising a nucleic acid vaccine of the present invention, which composition elicits an enhanced immune response, relative to controls, in the mammal against a clinical tumor related pathology.

In a further embodiment, the prophylactic or therapeutic method of eliciting an immune response to tumor comprising administering an effective amount of another (e.g., second) nucleic acid vaccine comprising at least 1 to about 100 different tumor protein fragments or variants, in which the fragments or variants relate to different tumor nucleic acid or amino sequences, preferably related to a cancer-associated or pathology-associated tumor protein or antigen sequence.

The tumor-specific immune response generated with at least one nucleic acid vaccine of the invention can be further augmented by priming or boosting a humoral or cellular immune response, or both, by administering an effective amount of at least one tumor/adjuvant vaccine. Any of the vaccine strategies provided herein or known in the art can be provided in any order. For example, a subject may be primed with a nucleic acid vaccine, followed by boosting with a nucleic acid vaccine or a protein vaccine. Preferably, the tumor/adjuvant vaccine is administered intramuscularly. Preferably, the vaccine is in the form of a plasmid and is administered with a gene gun or injector pen, needled or needleless. However, other forms and administration are also suitable and included in the present invention.

The present invention also provides methods, compositions, articles of manufacture and the like, for making and using a tumor/adjuvant nucleic acid vaccine of the present invention.

Other objects, features, advantages, utilities and embodiments of the present invention will be apparent to skilled practitioners from the following detailed description and examples relating to the present invention, in combination with what is known in the art.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Female C57Bl/6 mice were vaccinated three times (Day -28, -14, and -7) with buffer, empty vector, pMUC1 plasmid, pIL-18 plasmid, or combinations of the latter two plasmids. Animals were challenged with MUC1+ mouse tumor cells on Day 0, and were monitored for tumor incidence for 50 days.

Figure 2. Female C57Bl/6 mice were vaccinated three times (Day -28, -14 and -7) with buffer, empty vector, pMUC1 plasmid, pIL-18 plasmid, or combinations of the latter two plasmids. Animals were challenged with MUC1+ mouse tumor cells on Day 0, and were monitored for tumor growth for up to 50 days.

Figure 3. C57Bl/6 mice free of tumors in Figure 1 were rechallenged with MUC1⁺ tumor cells on Day 49 (denoted Day 0 in this figure). Mice were monitored an additional 49 days after the second tumor challenge.

Figure 4. MUC1 Tg mice were vaccinated three times (Day -28, -14, and -7) with the plasmids indicated in the legend. Mice were challenged with MUC1+ tumor cells on Day 0 and monitored for tumor incidence for 28 days.

Figure 5. Animals from Figure 4 were sacrificed, and their tumors were excised and weighed on Day 28 after tumor challenge. Horizontal bars are median values.

Figure 6. Phase II of the pMUC1/pIL-18 vaccination of MUC1 Tg mice. MUC1 Tg mice without tumors at the end of Phase I (Figure 4) were rechallenged with a second dose of MUC1+ tumor cells on Day 50 after the first challenge (denoted Day 0 in this figure). Mice were monitored for tumor incidence for 28 days after the second challenge.

Figure 7. Remaining tumor-free MUC1 Tg mice from Phase II (Figure 6) were challenged on Day 28 of Phase II with MUC1⁺ parental tumor cells (denoted as Day 0 in this figure). Animals were monitored for tumor incidence 39 days post challenge.

Figure 8A-C. A. DNA sequence of human IL-18 plasmid p1968 with the protein sequence of Figure 8B included. B, C. Protein sequence of the precursor human IL-18 produced by the engineered IL-18 constructs. The first 19 residues are derived from the 12B75 HC signal sequence; the remaining 161 residues are the mature human IL-18. In the version shown in C, the first residue of the mature human IL-18 sequence is altered to better conform to consensus human immunoglobulin signal sequences.

Figure 9A-D: Sequence of human MUC1 cDNA with intron 6 incorporated.

Figure 10. Tumor incidence in female MUC1 transgenic mice vaccinated with DNA as indicated in the legend, and subsequently challenged with MUC1⁺ tumor cells. Only the group vaccinated with pMUC1/pIL-18 shows significantly improved protection from tumor challenge ($p=0.007$).

Figure 11. Media tumor weights at study end, from animals shown in Figure 1. Media tumor weight for group 4 is significantly different from those in the other groups.

Figure 12. Rechallenge of protected mice from Figure 1 with MUC1⁺ tumor cells.

Figure 13. Tumor incidence in male mice vaccinated with pMUC1 or empty vector, followed by tumor challenge.

Figure 14. Tumor weights in male mice vaccinated with pMUC1.

Figure 15. Tumor incidence in male mice rechallenged on the opposite flank with MUC1⁺ tumor cells.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present inventors have discovered that unexpectedly enhanced immune responses can be induced against tumor associated pathologies, by the use of nucleic acid vaccines that contain a combination of at least one tumor antigen or protein encoding nucleic acid and at least one cytokine encoding nucleic acid.

The terms "priming" or "primary" and "boost" or "boosting" are used herein to refer to the initial and subsequent immunizations, respectively, i.e., in accordance with the definitions these terms normally have in immunology.

The component encoding nucleic acids of a tumor/adjuvant encoding nucleic acid of the present invention can be provided using any known method or source. Alternatively, the different tumor nucleic acids can be obtained from any source and selected based on screening of the sequences for differences in coding sequence or by evaluating differences in elicited humoral and/or cellular immune responses to multiple tumor sequences, in vitro or in vivo, according to known methods.

As is readily appreciated by one of skill in the art, the inventors have further found that boosting with a tumor/adjuvant vaccine of the present invention further potentiates the immunization methods of the invention. The tumor protein(s) encoded by the nucleic acid vaccine can be similar or different to the tumor protein(s) in the boosters.

Similarly, as can be appreciated by the skilled artisan, the immunization methods of the present invention are enhanced by use of primer, booster or additional administrations of a DNA vaccine of the present invention. The tumor/adjuvant vaccine can be used as a boost, e.g., as described above with respect to the tumor proteins. Alternatively, the vaccine can be used to prime immunity, with the vaccine or vaccines used to boost the anti-tumor immune response. The vaccine may comprise one or more vectors for expression of one or more tumor proteins or portions thereof. In a preferred embodiment, vectors are prepared for expression as part of a

DNA vaccine.

The invention is a therapeutic vaccine that would be used in patients with cancer, where PSA and/or KLK2 and/or MUC1 are uniquely expressed, or overexpressed relative to normal tissue. The vaccine could potentially be preventative therapy for individuals at high risk of developing prostate or other cancers or tumors expressing these antigens. The vaccine could also be used in other cancers where PSA and/or KLK2 and/or MUC1 are either uniquely expressed or overexpressed relative to normal tissue. The vaccine would be comprised of DNA encoding any combination of these antigens, and could be contained within one or more plasmids, mammalian viruses, bacteria or mammalian cells. The antigen or adjuvant encoding nucleic acids as one or more components of the vaccine could include any alternatively spliced forms that naturally occur. The antigen genes may contain modified sequences that will include optimized codons for translation in human cells, or signals for ubiquitination that would lead to enhanced degradation. The vaccine could contain fragments of the antigen genes, including antigen-specific CTL epitopes linked to each other, or to other heterologous CTL epitopes and/or homologous/heterologous CD4 helper epitopes. Fragments of the antigen genes could be generated that lack signal sequences, which could enhance degradation and antigen presentation. Fragments of the antigen genes could be encoded as fusions with other proteins, or inserted within other protein sequences, such as immunoglobulin sequences. Natural variant sequences have been reported for PSA, KLK2 and MUC1, and are useful in the present invention, e.g., but not limited to those presented in SEQ ID NOS:1-47, and specified variants thereof.

The vaccination regimen could include a mixture of DNA-encoding agents, temporally administered in different orders, or administered in different places in the body at the same time. Plasmids could be formulated in lipid, buffer or other excipients or chemical adjuvants that could aid delivery of DNA, maintain its integrity in vivo, or enhance the immunogenicity of the vaccine. The vaccine could also be delivered by direct injection into muscle, skin, lymph node, or by application to mucosal surfaces. Other potential modes of delivery would include injection of DNA, followed by electroporation to enhance cellular uptake and expression of DNA.

One possible cytokine adjuvant that could be included in the vaccine is human IL-18. Variants of human IL-18 sequence have been reported, , e.g., but not limited to those presented in SEQ ID NOS:60-77, and specified variants thereof. The macaque sequence for IL-18 is very similar to human IL-18, and can also be used according to the present invention.

The antigen genes, or costimulatory molecule genes, or cytokine adjuvant genes would be expressible in humans because of being linked to a promoter. The genes would also be expressible because of linkage to a polyadenylation signal, such as the SV40 late polyadenylation signal. An intron may be included for enhanced expression, such as the HCMV IE intronA, or natural introns from the antigen or adjuvant genes.

Advantages:

Active immunotherapy offers the possibility that cancer patients could develop long-lasting and vigorous immune responses against their tumors that would prolong life, slow disease progression, and possibly eradicate disease. When used as an adjunct therapy, active immunotherapy may increase quality of life by minimizing the toxicity of other conventional therapies. DNA vaccination in particular offers a simple approach toward generating protective immune responses.

We have demonstrated in our MUC1 vaccination model that DNA vaccination can lead to epitope spreading. There are no other reports of anti-tumor efficacy engendered by coadministration of plasmid DNA encoding MUC1 and any other costimulatory/adjuvant molecule, particularly IL-18. In addition, this is the only instance found so far of epitope spreading as a result of plasmid DNA vaccination in tumor models. As mentioned above, if this phenomenon could be induced in humans, it would induce immunity to MUC1 as well as to other unknown tumor-associated antigens that are present in the tumor. This multi-antigen attack on the tumor would minimize or inhibit the ability of the tumor to evade the immune response. This approach also is applicable to a vaccine using PSA as the antigen, or PSA in combination with other antigens and adjuvant molecules.

Another advantage of our approach is the ability to encode more than one gene on a plasmid or DNA vehicle to enable delivery of more than one protein product to a target tissue/cell (33, 34). This should ensure that a target tissue expresses all desired proteins with the expectation of a more efficient induction of immune response. For example, we have constructed a double cistron vector, and for example we have shown that it is capable of expressing mouse or human IL-12. IL-12 is a protein comprised of two subunits that must be co-expressed in the same cell in order for the mature molecule to be produced. The two protein subunits are encoded by different genes, and we have shown in tissue culture that a double cistron vector encoding both genes results in more effective production of the mature protein than using two plasmids which encode either gene alone (33, 34).

Nucleic acid vaccines and Vaccination

The present invention thus provides, in one aspect, nucleic acid vaccines using mixtures of at least 1, and up to 50 different tumor and cytokine encoding nucleic acids that optionally each can express a different protein variant, or an antigenic portion thereof. As can be readily appreciated to one of skill in the art, 1 to about 50 different tumor protein encoding nucleic acids can be employed. Also provided are methods of making and using such nucleic acid vaccines.

A nucleic acid vaccine of the present invention induces at least one of a humoral and a cellular immune response in a mammal who has been administered at least one nucleic acid vaccine, but the response to the vaccine is subclinical, or is effective in enhancing at least one immune response to at least one tumor antigen, such that the vaccine administration is suitable for vaccination purposes.

DNA vaccines. An alternative to a traditional vaccine comprising an antigen and an adjuvant involves the direct in vivo introduction of DNA encoding the antigen into tissues of a subject for expression of the antigen by the cells of the subject's tissue. Such vaccines are termed herein "DNA vaccines" or "nucleic acid-based vaccines." DNA vaccines are described in International Patent Publication WO 95/20660 and International Patent Publication WO 93/19183, the disclosures of which are hereby incorporated by reference in their entireties. The ability of directly injected DNA that encodes a viral protein to elicit a protective immune response has been demonstrated in numerous experimental systems (Conry et al., *Cancer Res.*, 54:1164-1168 (1994); Cox et al., *Virology*, 67:5664-5667 (1993); Davis et al., *Hum. Mole. Genet.*, 2:1847-1851 (1993); Sedegah et al., *Proc. Natl. Acad. Sci.*, 91:9866-9870 (1994); Montgomery et al., *DNA Cell Bio.*, 12:777-783 (1993); Ulmer et al., *Science*, 259:1745-1749 (1993); Wang et al., *Proc. Natl. Acad. Sci.*, 90:4156-4160 (1993); Xiang et al., *Virology*, 199:132-140 (1994)). Studies to assess this strategy in neutralization of influenza virus have used both envelope and internal viral proteins to induce the production of antibodies, but in particular have focused on the viral hemagglutinin protein (HA) (Fynan et al., *DNA Cell Biol.*, 12:785-789 (1993A); Fynan et al., *Proc. Natl. Acad. Sci.*, 90:11478-11482 (1993B); Robinson et al., *Vaccine*, 11:957, (1993); Webster et al., *Vaccine*, 12:1495-1498 (1994)).

As is well known in the art, a large number of factors can influence the efficiency of expression of antigen genes and/or the immunogenicity of DNA vaccines. Examples of such factors

include the reproducibility of inoculation, construction of the plasmid vector, choice of the promoter used to drive antigen gene expression and stability of the inserted gene in the plasmid. Depending on their origin, promoters differ in tissue specificity and efficiency in initiating mRNA synthesis (Xiang et al., *Virology*, 209:564-579 (1994); Chapman et al., *Nucle. Acids. Res.*, 19:3979-3986 (1991)). To date, most DNA vaccines in mammalian systems have relied upon viral promoters derived from cytomegalovirus (CMV). These have had good efficiency in both muscle and skin inoculation in a number of mammalian species. Another factor known to affect the immune response elicited by DNA immunization is the method of DNA delivery; parenteral routes can yield low rates of gene transfer and produce considerable variability of gene expression (Montgomery, 1993, *supra*). High-velocity inoculation of plasmids, using a gene-gun, enhanced the immune responses of mice (Fynan, 1993B, *supra*; Eisenbraun et al., *DNA Cell Biol.*, 12: 791-797 (1993)), presumably because of a greater efficiency of DNA transfection and more effective antigen presentation by dendritic cells. Vectors containing the nucleic acid-based vaccine of the invention may also be introduced into the desired host by other methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), or a DNA vector transporter (see, e.g., Wu et al., *J. Biol. Chem.* 267:963-967 (1992); Wu and Wu, *J. Biol. Chem.* 263:14621-14624 (1988); Hartmut et al., Canadian Patent Application No. 2,012,311, filed Mar. 15, 1990), or any other known method or device.

Viral Vector Vaccines. As can be readily appreciated by one of ordinary skill in the art, nucleic acid vaccines of the present invention can also be incorporated into any recombinant virus and can be used to introduce a vaccine of the invention. Examples of suitable viruses that can act as recombinant viral hosts for vaccines, in addition to vaccinia, includes canarypox, adenovirus, and adeno-associated virus, as known in the art. Various genetically engineered virus hosts ("recombinant viruses") can be used to prepare viral vaccines for administration of nucleic acid encoding tumor antigens. Viral vaccines can promote a suitable immune response that targets activation of B lymphocytes, helper T lymphocytes, and cytotoxic T lymphocytes. Numerous virus species can be used as the recombinant virus hosts for the vaccines of the invention. A preferred recombinant virus for a viral vaccine is vaccinia virus (International Patent Publication WO 87/06262, Oct. 22, 1987, by Moss et al.; Cooney et al., *Proc. Natl. Acad. Sci. USA* 90:1882-6 (1993); Graham et al., *J. Infect. Dis.* 166:244-52 (1992); McElrath et al., *J. Infect. Dis.* 169:41-7 (1994)). In another embodiment, recombinant canarypox can be used (Pialoux et al., *AIDS Res. Hum. Retroviruses* 11:373-81 (1995), erratum in *AIDS Res. Hum. Retroviruses* 11:875 (1995); Andersson et al., *J. Infect. Dis.* 174:977-85 (1996); Fries et al.,

Vaccine 14:428-34 (1996); Gonczol et al., Vaccine 13:1080-5 (1995)). Another alternative is defective adenovirus or adenovirus (Gilardi-Hebenstreit et al., J. Gen. Virol. 71:2425-31 (1990); Prevec et al., J. Infect. Dis. 161:27-30 (1990); Lubeck et al., Proc. Natl. Acad. Sci. USA 86:6763-7 (1989); Xiang et al., Virology 219:220-7 (1996)). Other suitable viral vectors include retroviruses that are packaged in cells with amphotropic host range (see Miller, Human Gene Ther. 1:5-14 (1990); Ausubel et al., Current Protocols in Molecular Biology, sec. 9), and attenuated or defective DNA virus, such as but not limited to herpes simplex virus (HSV) (see, e.g., Kaplitt et al., Molec. Cell. Neurosci. 2:320-330 (1991)), papillomavirus, Epstein Barr virus (EBV), adeno-associated virus (AAV) (see, e.g., Samulski et al., J. Virol. 61:3096-3101 (1987); Samulski et al., J. Virol. 63:3822-3828 (1989)), US Patent Nos: 5990091, 5766599, 5756103, 6086890, 6274147, 05585254, 6140114, 5616326, 6099847, 6221136, 6086891, 5958425, 5744143, 5558860, 5266489, 5858368, 5795872, 5693530, 6020172, and the like, each entirely incorporated herein by reference.

Bi-functional plasmids for virus and DNA vaccines. Another aspect of the present invention concerns engineering of bi-functional plasmids that can serve as a DNA vaccine and a recombinant virus vector. Direct injection of the purified plasmid DNA, i.e., as a DNA vaccine, would elicit an immune response to the antigen expressed by the plasmid in test subjects. The plasmid would also be useful in live, recombinant viruses as immunization vehicles.

The bi-functional plasmid of the invention provides a heterologous gene, or an insertion site for a heterologous gene, under control of two different expression control sequences: an animal expression control sequence, and a viral expression control sequence. The term "under control" is used in its ordinary sense, i.e., operably or operatively associated with, in the sense that the expression control sequence, such as a promoter, provides for expression of a heterologous gene. In another embodiment, the animal expression control sequence is a mammalian promoter (avian promoters are also contemplated by the present invention); in a specific embodiment, the promoter is a late or early SV40 promoter, cytomegalovirus immediate early (CMV) promoter, a vaccinia virus early promoter, or a vaccinia virus late promoter, or any combination thereof. Subjects could be vaccinated with a multi-tiered regimen, with the bi-functional plasmid administered as DNA and, at a different time, but in any order, as a recombinant virus vaccine. The invention contemplates single or multiple administrations of the bi-functional plasmid as a DNA vaccine or as a recombinant virus vaccine, or both. This vaccination regimen may be complemented with administration of viral vaccines (*infra*), or may be used with additional vaccine vehicles.

As one of ordinary skill in the art can readily appreciate, the bi-functional plasmids of the invention can be used as nucleic acid vaccine vectors. Thus, by inserting at least 1 to about 50 different tumor genes into bi-functional plasmids, thus preparing a corresponding set of bi-functional plasmids useful as a nucleic acid vaccine can be prepared.

Active immunity elicited by vaccination with a tumor protein or proteins according to the present invention can prime or boost a cellular or humoral immune response. The tumor protein or proteins, or antigenic fragments thereof, can be prepared in an admixture with an adjuvant to prepare a vaccine.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., Immunology, Second Ed., 1984, Benjamin/Cummings: Menlo Park, Calif., p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Selection of an adjuvant depends on the subject to be vaccinated. Preferably, a pharmaceutically acceptable adjuvant is used. For example, a vaccine for a human should avoid oil or hydrocarbon emulsion adjuvants, including complete and incomplete Freund's adjuvant. One example of an adjuvant suitable for use with humans is alum (alumina gel). In a specific embodiment, recombinant tumor protein is administered intramuscularly in alum. Alternatively, the recombinant tumor protein vaccine can be administered subcutaneously, intradermally, intraperitoneally, or via other acceptable vaccine administration routes.

Vaccine administration. According to the invention, immunization against tumors can be accomplished with a nucleic acid tumor/adjuvant vaccine of the invention alone, or in combination with a viral encoding tumor vaccine or a tumor protein vaccine, or both. In a specific embodiment, tumor nucleic acid or viral vaccine is provided intramuscularly (i.m.) to boost the immune response.

Each dose of vaccine may contain the same 1 to 50 nucleic acid sequences encoding the same

or different tumor proteins or portions thereof. Alternatively, the tumor sequences in subsequent vaccines may express different tumor genes or portions thereof. In yet another embodiment, the subsequent vaccines may have some tumor sequences in common, and others that are different, from the earlier vaccine. For example, the priming vaccine may contain nucleic acids expressing tumor proteins arbitrarily designated 1-2. A second (booster) vaccine may contain vaccines expressing tumor proteins 3-5 or 6-10, etc.

Tumor Vaccine Variants

As noted above, a tumor/adjuvant encoding nucleic acid for use in the vaccines of the invention can be obtained from different cancer or normal tumor patients or different geographically local isolates, or from geographically diverse isolates.

A tumor/adjuvant vaccine also includes nucleic acid encoding polypeptides having immunogenic activity elicited by an amino acid sequence of a tumor amino acid sequence as at least one epitope or antigenic determinant. Such amino acid sequences substantially correspond to at least one 10-200 amino acid fragment and/or consensus sequence of a known tumor antigen protein sequence, as described herein or as known in the art. Such a tumor antigen sequence can have overall homology or identity of at least 50% to a known tumor protein amino acid sequence, such as 50-99% homology, or any range or value therein, while eliciting an immunogenic response against at least one type of tumor protein, preferably including at least one pathologic form.

Percent homology can be determined, for example, by comparing sequence information using the GAP computer program, version 6.0. available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443 (1970)), as revised by Smith and Waterman (Adv. Appl. Math. 2:482 (1981)). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unitary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov and Burgess, Nucl. Acids Res. 14:6745 (1986), as described by Schwartz and Dayhoff, eds., Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington, D.C. (1979), pp. 353-358; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

In another embodiment, a tumor/adjuvant vaccine of the present invention comprises a pathologic form of at least one tumor protein. Examples of such sequences are readily available from commercial and institutional tumor sequence databases, such as GENBANK, or other publically available databases. Substitutions or insertions of a tumor or cytokine to obtain an additional tumor or cytokine protein, encoded by a nucleic acid for use in a viral or nucleic acid vaccine of the present invention, can include substitutions or insertions of at least one amino acid residue (e.g., 1-25 amino acids). Alternatively, at least one amino acid (e.g., 1-25 amino acids) can be deleted from a tumor or cytokine sequence. Preferably, such substitutions, insertions or deletions are identified based on sequence determination of proteins obtained by nucleotide sequencing of at least one tumor or cytokine encoding nucleic acid from an individual.

Non-limiting examples of such substitutions, insertions or deletions preferably are made by the amplification of DNA or RNA sequences from tumor, which can be determined by routine experimentation to provide modified structural and functional properties of a protein or a tumor or cytokine. The tumor or cytokine protein sequences so obtained preferably have different antigenic or adjuvant properties from the original tumor or cytokine. Such antigenic differences can be determined by suitable assays, e.g., by testing with a panel of monoclonal antibodies specific for tumor or cytokine proteins in an ELISA assay.

Any substitution, insertion or deletion can be used as long as the resulting tumor and cytokine proteins or antigenic determinants thereof elicits antibodies which bind to tumor proteins, but which tumor proteins have a different pattern than antibodies elicited by a second tumor protein. Each of the above substitutions, insertions or deletions can also include modified or unusual amino acids, e.g., as provided in 37 C.F.R. section 1.822(p)(2), which is entirely incorporated herein by reference.

The following present non-limiting examples of alternative nucleic acid sequences (recited as DNA sequences, but also including the corresponding RNA sequence (where U is substituted for T in the corresponding RNA sequence)) of tumor antigen proteins of tumors, as well as cytokine adjuvant nucleic acid sequences, that can be encoded by a nucleic acid according to present invention. Such nucleic acid vaccines can comprise at least one tumor antigen protein encoding nucleic acid and at least one cytokine adjuvant protein encoding nucleic acid, and can include linear or circular DNA or RNA, optionally further comprising additional regulatory sequences, such as but not limited to promoters, enhancers, selection, restriction sites, and the

like, as well known in the art. For amino acid sequences any suitable codon can be used for expression, preferably human preferred codons as well known in the art (see, e.g., Ausubel, supra, Appendices) and such sequences can be further modified, e.g., where specific antigenic sequences can be used.

SEQUENCE LISTING

PSA/KLK3 sequences

1. PSA (SEQ ID NO:1)

Ile	Val	Gly	Gly	Trp	Glu	Cys	Glu	Lys	His	Ser	Gln	Pro	Trp	Gln	Val	1	5	10	15
Leu	Val	Ala	Ser	Arg	Gly	Arg	Ala	Val	Cys	Gly	Gly	Val	Leu	Val	His	20	25	30	
Pro	Gln	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Ile	Arg	Asn	Lys	Ser	Val	35	40	45	
Ile	Leu	Leu	Gly	Arg	His	Ser	Leu	Phe	His	Pro	Glu	Asp	Thr	Gly	Gln	50	55	60	
Val	Phe	Gln	Val	Ser	His	Ser	Phe	Pro	His	Pro	Leu	Tyr	Asp	Met	Ser	65	70	75	80
Leu	Leu	Lys	Asn	Arg	Phe	Leu	Arg	Pro	Gly	Asp	Asp	Ser	Ser	His	Asp	85	90	95	
Leu	Met	Leu	Leu	Arg	Leu	Ser	Glu	Pro	Ala	Glu	Leu	Thr	Asp	Ala	Val	100	105	110	
Lys	Val	Met	Asp	Leu	Pro	Thr	Gln	Glu	Pro	Ala	Leu	Gly	Thr	Thr	Cys	115	120	125	
Tyr	Ala	Ser	Gly	Trp	Gly	Ser	Ile	Glu	Pro	Glu	Glu	Phe	Leu	Thr	Pro	130	135	140	
Lys	Lys	Leu	Gln	Cys	Val	Asp	Leu	His	Val	Ile	Ser	Asn	Asp	Val	Cys	145	150	155	160
Ala	Gln	Val	His	Pro	Gln	Lys	Val	Thr	Lys	Phe	Met	Leu	Cys	Ala	Gly	165	170	175	
Arg	Trp	Thr	Gly	Gly	Lys	Ser	Thr	Cys	Ser	Gly	Asp	Ser	Gly	Gly	Pro	180	185	190	
Leu	Val	Cys	Asn	Gly	Val	Leu	Gln	Gly	Ile	Thr	Ser	Trp	Gly	Ser	Glu	195	200	205	

Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr Thr Lys Val Val His
210 215 220

Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala Asn Pro
225 230 235

PSA 1: human PSA with introns (SEQ ID NO:2):

gtccgtgacg tggattggtg ctgcacccct catcctgtct cggattgtgg
gaggctggga 60
gtgcgagaag cattcccaac cctggcaggt gcttgtggcc tctcgtggca gggcagtcctg 120
cggcggtggt ctggtgcacc cccagtgggt cctcacagct gccactgca tcaggaacaa 180
aagcgtgac ttgctgggtc ggcacagcct gtttcacct gaagacacag gccagggtatt 240
tcagggtcagc cacagcttcc cacacccgct ctacgatatg agcctcctga agaatcgatt 300
cctcaggcca ggtgatgact ccagccacga cctcatgctg ctccgcctgt cagagcctgc 360
cgagctcag gatgctgtga aggtcatgga cctgcccacc caggagccag cactggggac 420
cacctgctac gcctcaggct ggggcagcat tgaaccagag gaggttctga ccccaaagaa 480
acttcagtgt gtggacctcc atgttatttc caatgacgtg tgtgcgcaag ttcacctca 540
gaaggtgacc aagttcatgc tgtgtgtgg acgttgaca gggggcaaaa gcacctgctc 600
gggtgattct gggggcccac ttgtctgtaa tgggtgtgct caaggtatca cgtcatgggg 660
cagtgaacca tgtgccctgc ccgaaaggcc ttccctgtac accaagggtg tgcattaccg 720
gaagtggatc aaggacacca tctgtggcaa cccctgagca cccctatcaa cccctattg 780
tagtaaacctt ggaaccttgg aatgaccag gccaaagactc aagcctcccc agttctactg 840
acctttgtcc ttaggtgtga ggtccagggt tgctaggaag aaaaatcagc agacacaggt 900
gtagaccaga gtgtttctta aatggtgtaa tttgtctc tctgtgtcct ggggaatact 960
ggccatgcct ggagacatat cactcaattt ctctgaggac acagatagga tggggtgtct 1020
gtgttatttg tggggtacag agatgaaaga ggggtgggat ccacactgag agagtggaga 1080
gtgacatgtg ctggacactg tccatgaagc actgagcaga agctggaggc acaacgcacc 1140
agacactcac agcaaggatg gagctgaaaa cataaccac tctgtcctgg aggcactggg 1200
aagcctagag aaggctgtga gccaaaggag gaggtcttc ctttggcatg ggatggggat 1260
gaagtaagga gagggactgg acccctgga agctgattca ctatgggggg aggtgtattg 1320
aagtcctcca gacaaccctc agatttgatg atttctagt agaactcaca gaaataaaga 1380
tgtg 1394

2. PSA 2: SEQ ID NO:1, comprising one or more or any combination of Thr40, Met112, and/or deletion of one or more of Tyr225, Arg226, Lys227, Trp228, Ile229, Lys230, Asp231, Thr232, Ile233, Val234, Ala235, Asn236, Pro237.

3. PSA 3: cDNA sequence with introns (SEQ ID NO:3):

aagtttccct tctcccagtc caagacccca aatcaccaca aaggacccaa tcccagact 61
caagatatgg tctgggcgct gtcttgtgtc tcctaccctg atccctgggt tcaactctgc 121
tcccagagca tgaagcctct ccaccagcac cagccaccaa cctgcaaacc tagggaagat 181
tgacagaatt cccagccttt cccagctccc cctgcccctg tcccaggact cccagccttg 241
gttctctgcc cccgtgtctt ttcaaaccce catcctaaat ccattctcta tccgagtcctc 301
ccagttcctc ctgtcaaccc tgattccctt gatctagcac cccctctgca ggtgctgcac 361
ccctcatcct gtctcggatt gtgggaggct gggagtgcga gaagcattcc caaccctggc 421
aggtgcttgt agcctctcgt ggcagggcag tctgcggcgg tgttctggtg caccctcagt 481
gggtcctcac agctacccac tgcatacagga acaaaagcgt gatcttgctg ggtcggcaca 541
gcctgtttca tctgaagac acaggccagg tatttcagggt cagccacagc ttcccacacc 601
cgctctacga tatgagcctc ctgaagaatc gattcctcag gccagggtgat gactccagcc 661
acgacctcat gctgctccgc ctgctagagc ctgccagct cacggatgct atgaagggtca 721
tggacctgcc caccaggag ccagcactgg ggaccacctg ctacgcctca ggctggggca 781
gcattgaacc agaggagtgc ttgaccccaa agaaacttca gtgtgtggac ctccatgtta 841
tttccaatga cgtgtgtgog caagtccacc ctgagaagg gaccaagtgc atgctgtgtg 901
ctggacgctg gacagggggc aaaagcacct gctcgggtga ttctgggggg ccacttgtct 961
gtaatggtgt gcttcaagggt atcacgtcat ggggcagtg accatgtgcc ctgcccga 1021
ggccttcctc gtacaccaag gtggtgcatt accggaagt gatcaaggac accatcgtgg 1081

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ccaacccctg agcacccta tcaactccct attgtagtaa acttggaacc ttggaaatga 1141
ccaggccaag actcagccct cccagttct actgacctt gtccttaggt gtgaggtcca 1201
gggttgctag gaaaagaaat cagcagacac aggtgtagac cagagtgttt cttaaattgt 1261
gtaattttgt cctctctgtg tcttggggaa tactggccat gcttggagac atatcactca 1321
atttctctga ggacacagat aggatggggt gtctgtgtta tttgtggggt acagagatga 1381
aagaggggtg ggatccacac tgagagagtg gagagtgaca tgtgctggac actgtccatg 1441
aagcactgag cagaagctgg aggcacaacg caccagacac tcacagcaag gatggagctg 1501
aaaacataac ccactctgtc ctggaggcac tgggaagcct agagaaggct gtgaaccaag 1561
gagggagggt cttcctttgg catgggatgg ggatgaagta aggagagggg ctgacccct 1621
ggaagctgat tcactatggg gggaggtgta ttgaagtcc cagacaacc ctgagatttg 1681
atgatttcct agtagaactc acagaaataa agagctgtta tactgtgaa

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3. rhesus macaque PSA (SEQ ID NO:4):

```

Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1           5           10           15

```

```

Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly Val Leu Val His
          20           25           30

```

```

Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg Ser Asn Ser Val
          35           40           45

```

```

Ile Leu Leu Gly Arg His Asn Pro Tyr Tyr Pro Glu Asp Thr Gly Gln
50           55           60

```

```

Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu Tyr Asn Met Ser
65           70           75           80

```

```

Leu Leu Lys Asn Arg Tyr Leu Gly Pro Gly Asp Asp Ser Ser His Asp
          85           90           95

```

```

Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Ile Thr Asp Ala Val
          100          105          110

```

```

Gln Val Leu Asp Leu Pro Thr Trp Glu Pro Glu Leu Gly Thr Thr Cys
          115          120          125

```

```

Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu His Leu Thr Pro
          130          135          140

```

```

Lys Lys Leu Gln Cys Val Asp Leu His Ile Ile Ser Asn Asp Val Cys
          145          150          155          160

```

```

Ala Gln Val His Ser Gln Lys Val Thr Lys Phe Met Leu Cys Ala Gly
          165          170          175

```

```

Ser Trp Met Gly Gly Lys Ser Thr Cys Ser Gly Asp Ser Gly Gly Pro
          180          185          190

```

```

Leu Val Cys Asp Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Ser Gln
          195          200          205

```

```

Pro Cys Ala Leu Pro Arg Arg Pro Ser Leu Tyr Thr Lys Val Val Arg
          210          215          220

```

Tyr Arg Lys Trp Ile Gln Asp Thr Ile Met Ala Asn Pro
 225 230 235

PSA 4: rhesus PSA : SEQ ID NO:4, comprising one or more or any combination of Thr40, Met112, and/or deletion of one or more of Tyr225, Arg226, Lys227, Trp228, Ile229, Gln230, Asp231, Thr232, Ile233, Met234, Ala235, Asn236, Pro237.

4. CTL epitopes from PSA

PSA antigen SEQ ID NO:5:

Phe Leu Thr Pro Lys Lys Leu Gln Cys Val
 1 5 10

PSA antigen SEQ ID NO:6:

Lys Leu Gln Cys Val Asp Leu His Val
 1 5

PSA antigen SEQ ID NO:7

Val Ile Ser Asn Asp Val Cys Ala Gln Val
 1 5 10

PSA antigen SEQ ID NO:8

Val Leu Val His Pro Gln Trp Val Leu
 1 5

PSA antigen SEQ ID NO:9

Gln Val His Pro Gln Lys Val Thr Lys
 1 5

5. PSA antigen SEQ ID NO:10:

Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly Ala Ala Pro Leu
 1 5 10 15

Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln
 20 25 30

Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly
 35 40 45

Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg
 50 55 60

Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu
 65 70 75 80

Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu
 85 90 95

Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp

PSA cDNA (SEO ID NO:11)

ggtgtgtcttc	ctcaccctgt	cgtgacgtg	gattggtgct	gcaccctca	tctgtctctg	60
gattgtggga	ggctgggagt	gcgagaagca	ttcccaaccc	tggcaggtgc	ttgtggcctc	120
tcgtggcagg	gcagtctgcg	gcggtgttct	ggtgcacccc	cagtgggtcc	tcacagctgc	180
ccactgcata	aggaacaaaa	gcgtgatctt	gctgggtcgg	cacagcctgt	ttcatcctga	240
agacacaggc	caggtatttc	aggtcagcca	cagcttcccc	caccgcctct	acgatatgag	300
cctcctgaag	aatcgatttc	tcaggccagg	tgtactgctc	agccagcacc	tcatgtgctc	360
cgcctgtca	gagcctgccg	agctcacgga	tgctgtgaag	gtcatggacc	tgccaccca	420
ggagccagca	ctggggacca	cctgctacgc	ctcaggctgg	ggcagcattg	aaccagagga	480
gttcttgacc	ccaaagaaac	ttcagtgtgt	ggacctccat	gttatttcca	atgacgtgtg	540
tgcgcaagtt	cacctcaga	aggtgaccaa	gttcattgctg	tgtgctggac	gctggacagg	600
gggcaaaagc	acctgctcgt	gggtcattct	gatcaccgaa	ctgaccatgc	cagccctgcc	660
gatggctcct	catggctccc	tagtgcctcg	gagaggaggt	gtctagtctg	agagtgtgcc	720
tggaaaggtg	cctctgtgag	gagccacggg	gacagcatcc	tgcagatggt	cctggccctt	780
gtccacccga	cctgtctaca	aggactgtcc	tcgtggaccc	tccctctggt	acaggagctg	840
gacctgaag	tcccttccct	accggccagg	actggagccc	ctaccctctc	gttggaatcc	900
ctgcccacct	tcttctggaa	gtcggctctg	gagacatttc	tctcttcttc	caaagctggg	960
aactgctatc	tgttatctgc	ctgtccaggt	ctgaaagata	ggattgccca	ggcagaaact	1020
gggactgacc	tatctcactc	tctccttctc	tttacctta	gggtgattct	ggggggccac	1080
ttgtctgtaa	tggtgtgctt	caaggtatca	cgtcatgggg	cagtgaacca	tgtgccctgc	1140
ccgaaaggta	tccctgtgac	accaaggtgg	tgcattaccg	gaagtgatc	aaggacacca	1200
tcgtggccaa	ccccgtagca	cccctatcaa	ctccctattg	tagtaaacct	ggaaccttgg	1260
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cactcaattt	ctctgaggac	acagatagga	tgggttgtct	gtgttatttg	tggggtagag	1500
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gtccatgaag	cactgagcag	aagctggagg	cacaacgcac	cagacactca	cagcaaggat	1620
ggaactgaaa	acataaccca	ctctgtcctg	gaag			1654

PSA ANTIGEN AA SEQ ID NO: 12

Val Val Phe Leu Thr Leu Ser Val Thr Trp Ile Gly Ala Ala Pro Leu
 1 5 10 15
 Ile Leu Ser Arg Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln
 20 25 30
 Pro Trp Gln Val Leu Val Ala Ser Arg Gly Arg Ala Val Cys Gly Gly
 35 40 45
 Val Leu Val His Pro Gln Trp Val Leu Thr Ala Ala His Cys Ile Arg
 50 55 60
 Asn Lys Ser Val Ile Leu Leu Gly Arg His Ser Leu Phe His Pro Glu
 65 70 75 80
 Asp Thr Gly Gln Val Phe Gln Val Ser His Ser Phe Pro His Pro Leu
 85 90 95
 Tyr Asp Met Ser Leu Leu Lys Asn Arg Phe Leu Arg Pro Gly Asp Asp
 100 105 110
 Ser Ser His Asp Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Glu Leu
 115 120 125
 Thr Asp Ala Val Lys Val Met Asp Leu Pro Thr Gln Glu Pro Ala Leu
 130 135 140
 Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu
 145 150 155 160
 Cys Thr Pro Gly Pro Asp Gly Ala Ala Gly Ser Pro Asp Ala Trp Val
 165 170 175

PSA ANTIGEN DNA SEQ ID NO:13

gggtgtcttc ctcacctgt ccgtgacgtg gattgggtgct gcacccctca
 tcctgtctcg 60
 gattgtggga ggctgggagt gcgagaagca ttcccaaccc tggcaggtgc
 ttgtggcctc 120
 tcgtggcagg gcagtctgcg gcggtgttct ggtgcacccc cagtgggtcc
 tcacagctgc 180
 ccactgcctc aggaacaaaa gcgtgatctt gctgggtcgg cacagcctgt
 ttcctcctga 240
 agacacaggc caggtatttc aggtcagcca cagcttccca caccgctct
 acgatatgag 300
 cctcctgaag aatcgattcc tcaggccagg tgatgactcc agccacgacc
 tcatgctgct 360
 ccgctgtca gagcctgccg agctcacgga tgctgtgaag gtcattggacc
 tgccaccca 420
 ggagccagca ctggggacca cctgctacgc ctcaggctgg ggcagcattg
 aaccagagga 480
 gtgtacgcct gggccagatg gtgcagccgg gagcccagat gcctgggtct
 gagggaggag 540

gggacaggac tcctgggtct gaggaggagg ggccaaggaa ccaggtgggg
 tccagccac 600
 aacagtgttt ttgcctggc ccgtagtctt gaccccaaag aaacttcagt gtgtggac
 658

PSA ANTIGEN AA SEQ ID NO:14

Val	Val	Phe	Leu	Thr	Leu	Ser	Val	Thr	Trp	Ile	Gly	Ala	Ala	Pro	Leu
1				5					10					15	
Ile	Leu	Ser	Arg	Ile	Val	Gly	Gly	Trp	Glu	Cys	Glu	Lys	His	Ser	Gln
			20					25					30		
Pro	Trp	Gln	Val	Leu	Val	Ala	Ser	Arg	Gly	Arg	Ala	Val	Cys	Gly	Gly
		35					40					45			
Val	Leu	Val	His	Pro	Gln	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Ile	Arg
	50					55					60				
Asn	Lys	Ser	Val	Ile	Leu	Leu	Gly	Arg	His	Ser	Leu	Phe	His	Pro	Glu
65					70					75					80
Asp	Thr	Gly	Gln	Val	Phe	Gln	Val	Ser	His	Ser	Phe	Pro	His	Pro	Leu
			85						90					95	
Tyr	Asp	Met	Ser	Leu	Leu	Lys	Asn	Arg	Phe	Leu	Arg	Pro	Gly	Asp	Asp
			100					105					110		
Ser	Ser	His	Asp	Leu	Met	Leu	Leu	Arg	Leu	Ser	Glu	Pro	Ala	Glu	Leu
			115				120					125			
Thr	Asp	Ala	Val	Lys	Val	Met	Asp	Leu	Pro	Thr	Gln	Glu	Pro	Ala	Leu
	130					135					140				
Gly	Thr	Thr	Cys	Tyr	Ala	Ser	Gly	Trp	Gly	Ser	Ile	Glu	Pro	Glu	Glu
145					150					155					160
Cys	Thr	Pro	Gly	Pro	Asp	Gly	Ala	Ala	Gly	Ser	Pro	Asp	Ala	Trp	Val
			165						170					175	

PSA ANTIGEN AA SEQ ID NO:15

Ile	Val	Gly	Gly	Trp	Glu	Cys	Glu	Lys	His	Ser	Gln	Pro	Trp	Gln	Val
1				5					10					15	
Leu	Val	Ala	Ser	Arg	Gly	Arg	Ala	Val	Cys	Gly	Gly	Val	Leu	Val	His
			20					25					30		
Pro	Gln	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Ile	Arg	Lys	Pro	Gly	Asp
		35					40					45			
Asp	Ser	Ser	His	Asp	Leu	Met	Leu	Leu	Arg	Leu	Ser	Glu	Pro	Ala	Glu
	50					55					60				
Leu	Thr	Asp	Ala	Val	Lys	Val	Met	Asp	Leu	Pro	Thr	Gln	Glu	Pro	Ala
65					70					75					80

Leu Gly Thr Thr Cys Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu
 85 90 95
 Glu Phe Leu Thr Pro Lys Lys Leu Gln Cys Val Asp Leu His Val Ile
 100 105 110
 Ser Asn Asp Val Cys Ala Gln Val His Pro Gln Lys Val Thr Lys Phe
 115 120 125
 Met Leu Cys Ala Gly Arg Trp Thr Gly Gly Lys Ser Thr Cys Ser Gly
 130 135 140
 Asp Ser Gly Gly Pro Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr
 145 150 155 160
 Ser Trp Gly Ser Glu Pro Cys Ala Leu Pro Glu Arg Pro Ser Leu Tyr
 165 170 175
 Thr Lys Val Val His Tyr Arg Lys Trp Ile Lys Asp Thr Ile Val Ala
 180 185 190
 Asn Pro

II. KLK2 sequences

KLK2 AA SEQ ID NO:16

Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
 1 5 10 15
 Ala Val Tyr Ser His Gly Trp Ala His Cys Gly Gly Val Leu Val His
 20 25 30
 Pro Gln Trp Val Leu Thr Ala Ala His Cys Leu Lys Lys Asn Ser Gln
 35 40 45
 Val Trp Leu Gly Arg His Asn Leu Phe Glu Pro Glu Asp Thr Gly Gln
 50 55 60
 Arg Val Pro Val Ser His Ser Phe Pro His Pro Leu Tyr Asn Met Ser
 65 70 75 80
 Leu Leu Lys His Gln Ser Leu Arg Pro Asp Glu Asp Ser Ser His Asp
 85 90 95
 Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Lys Ile Thr Asp Val Val
 100 105 110
 Lys Val Leu Gly Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
 115 120 125
 Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Arg Pro
 130 135 140
 Arg Ser Leu Gln Cys Val Ser Leu His Leu Leu Ser Asn Asp Met Cys
 145 150 155 160
 Ala Arg Ala Tyr Ser Glu Lys Val Thr Glu Phe Met Leu Cys Ala Gly

	165		170		175
Leu Trp Thr Gly Gly Lys Asp Thr Cys Gly Gly Asp Ser Gly Gly Pro					
	180		185		190
Leu Val Cys Asn Gly Val Leu Gln Gly Ile Thr Ser Trp Gly Pro Glu					
	195		200		205
Pro Cys Ala Leu Pro Glu Lys Pro Ala Val Tyr Thr Lys Val Val His					
	210		215		220
Tyr Arg Lys Trp Ile Lys Asp Thr Ile Ala Ala Asn Pro					
	225		230		235

KLK2 DNA SEQ ID NO:17

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gctggatgtg gtggtgcatg cttgtggtct cagctatcct ggaggctgag acaggagaat 60
cggttgagtc tgggagttca aggctacagg gagctgogat cacgcegtg cactccagcc 120
tgggaaacag agtgagactg tctcagaatt tttttaaaaa agaatacagt atcatcccaa 180
ccccgtttgc tgttcacact gagcctgcct tctctggctt tgttccctag atcacatctc 240
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tagtatgtgt ggaacagcaa gtgctggctc tcctccctc tcacagctc tgggtgtggg 360
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gaaatgggta	taatttcgtc	ctctccttcg	gaacactggc	tgtctctgaa	gacttctcgc	5880
tcagtttcag	tgaggacaca	cacaaagacg	tgggtgacca	tgttgtttgt	ggggtgcaga	5940
gatgggaggg	gtggggcca	cctgggaagag	tggcagtgga	cacaaggtgg	acactctcta	6000
cagatcactg	aggataagct	ggagccacaa	tgcattgaggc	acacacacag	caaggatgac	6060
gctgtaaaaca	tagccacgc	tgtcctgggg	gcactgggaa	gcctagataa	ggccgtgagc	6120
agaaagaagg	ggaggtacc					6139

human KLK2 AA SEQ ID NO:18

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Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1           5           10           15
Ala Val Tyr Ser His Gly Trp Ala His Cys Gly Gly Val Leu Val His
          20           25           30
Pro Gln Trp Val Leu Thr Ala Ala His Cys Leu Lys Lys Asn Ser Gln
          35           40           45
Val Trp Leu Gly Arg His Asn Leu Phe Glu Pro Glu Asp Thr Gly Gln
50           55           60
Arg Val Pro Val Ser His Ser Phe Pro His Pro Leu Tyr Asn Met Ser
65           70           75           80
Leu Leu Lys His Gln Ser Leu Arg Pro Asp Glu Asp Ser Ser His Asp
          85           90           95
Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Lys Ile Thr Asp Val Val
          100          105          110
Lys Val Leu Gly Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
          115          120          125
Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu
130          135          140

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human KLK2 AA SEQ ID NO:19

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Ile Val Gly Gly Trp Glu Cys Glu Lys His Ser Gln Pro Trp Gln Val
1           5           10           15
Ala Val Tyr Ser His Gly Trp Ala His Cys Gly Gly Val Leu Val His
          20           25           30
Pro Gln Trp Val Leu Thr Ala Ala His Cys Leu Lys Lys Asn Ser Gln
          35           40           45
Val Trp Leu Gly Arg His Asn Leu Phe Glu Pro Glu Asp Thr Gly Gln
50           55           60
Arg Val Pro Val Ser His Ser Phe Pro His Pro Leu Tyr Asn Met Ser
65           70           75           80
Leu Leu Lys His Gln Ser Leu Arg Pro Asp Glu Asp Ser Ser His Asp
          85           90           95
Leu Met Leu Leu Arg Leu Ser Glu Pro Ala Lys Ile Thr Asp Val Val
          100          105          110
Lys Val Leu Gly Leu Pro Thr Gln Glu Pro Ala Leu Gly Thr Thr Cys
          115          120          125
Tyr Ala Ser Gly Trp Gly Ser Ile Glu Pro Glu Glu Phe Leu Arg Pro
130          135          140

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Arg Ser Leu Gln Cys Val Ser Leu His Leu Leu Ser Asn Asp Met Cys
 145 150 155 160

Ala Arg Ala Tyr Ser Glu Lys Val Thr Glu Phe Met Leu Cys Ala Gly
 165 170 175

Leu Trp Thr Gly Gly Lys Asp Thr Cys Gly Val Ser His Pro Tyr Ser
 180 185 190

Gln His Leu Glu Gly Lys Gly
 195

III. MUC1 Sequences

human MUC1 AA: (SEQ ID NO:20)

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Leu Thr
 1 5 10 15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
 20 25 30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
 35 40 45

Thr Glu Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His
 50 55 60

Ser Pro Gly Ser Gly Ser Ser Thr Thr Gln Gly Gln Asp Val Thr Leu
 65 70 75 80

Ala Pro Ala Thr Glu Pro Ala Ser Gly Ser Ala Ala Thr Trp Gly Gln
 85 90 95

Asp Val Thr Ser Val Pro Val Thr Arg Pro Ala Leu Gly Ser Thr Thr
 100 105 110

Pro Pro Ala His Asp Val Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro
 115 120 125

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
 130 135 140

Arg Pro Pro Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
 145 150 155 160

Ala Pro Asp Thr Arg Pro Pro Pro Gly Ser Thr Ala Pro Ala Ala His
 165 170 175

Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala
 180 185 190

Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Asn Arg Pro Ala Leu
 195 200 205

Ala Ser Thr Ala Pro Pro Val His Asn Val Thr Ser Ala Ser Gly Ser
 210 215 220

Ala Ser Gly Ser Ala Ser Thr Leu Val His Asn Gly Thr Ser Ala Arg
 225 230 235 240

Ala Thr Thr Thr Pro Ala Ser Lys Ser Thr Pro Phe Ser Ile Pro Ser
 245 250 255
 His His Ser Asp Thr Pro Thr Thr Leu Ala Ser His Ser Thr Lys Thr
 260 265 270
 Asp Ala Ser Ser Thr His His Ser Thr Val Pro Pro Leu Thr Ser Ser
 275 280 285
 Asn His Ser Thr Ser Pro Gln Leu Ser Thr Gly Val Ser Phe Phe Phe
 290 295 300
 Leu Ser Phe His Ile Ser Asn Leu Gln Phe Asn Ser Ser Leu Glu Asp
 305 310 315 320
 Pro Ser Thr Asp Tyr Tyr Gln Glu Leu Gln Arg Asp Ile Ser Glu Met
 325 330 335
 Phe Leu Gln Ile Tyr Lys Gln Gly Gly Phe Leu Gly Leu Ser Asn Ile
 340 345 350
 Lys Phe Arg Pro Gly Ser Val Val Val Gln Leu Thr Leu Ala Phe Arg
 355 360 365
 Glu Gly Thr Ile Asn Val His Asp Val Glu Thr Gln Phe Asn Gln Tyr
 370 375 380
 Lys Thr Glu Ala Ala Ser Arg Tyr Asn Leu Thr Ile Ser Asp Val Ser
 385 390 395 400
 Val Ser Asp Val Pro Phe Pro Phe Ser Ala Gln Ser Gly Ala Gly Val
 405 410 415
 Pro Gly Trp Gly Ile Ala Leu Leu Val Leu Val Cys Val Leu Val Ala
 420 425 430
 Leu Ala Ile Val Tyr Leu Ile Ala Leu Ala Val Cys Gln Cys Arg Arg
 435 440 445
 Lys Asn Tyr Gly Gln Leu Asp Ile Phe Pro Ala Arg Asp Thr Tyr His
 450 455 460
 Pro Met Ser Glu Tyr Pro Thr Tyr His Thr His Gly Arg Tyr Val Pro
 465 470 475 480
 Pro Ser Ser Thr Asp Arg Ser Pro Tyr Glu Lys Val Ser Ala Gly Asn
 485 490 495
 Gly Gly Ser Ser Leu Ser Tyr Thr Asn Pro Ala Val Ala Ala Thr Ser
 500 505 510
 Ala Asn Leu
 515

MUC1 DNA sequence: (SEQ ID NO:21)

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 acaggttctg gtcattgcaag ctctacccca ggtggagaaa aggagacttc ggctacccag 180
 agaagttcag tgcccagctc tactgagaag aatgctgtga gtatgaccag cagcgtactc 240


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ccggacaaca agccagcccc ggggtccacc gccccccag cccacgggtgt cacctcggcc 480
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ctgggagccc ccaccacaac acttcccagg catggaattc c 1721

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1. Complete coding sequence of MUC1 (genomic and protein translation, but does not include complete set of tandem repeats, probably in interest of space)
: (SEQ ID NO:22)

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Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1          5          10          15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
20        25        30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
35        40        45

Thr Glu Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His
50        55        60

Ser Pro Gly Ser Gly Ser Ser Thr Thr Gln Gly Gln Asp Val Thr Leu
65        70        75        80

Ala Pro Ala Thr Glu Pro Ala Ser Gly Ser Ala Ala Thr Trp Gly Gln
85        90        95

Asp Val Thr Ser Val Pro Val Thr Arg Pro Ala Leu Gly Ser Thr Thr
100       105       110

Pro Pro Ala His Asp Val Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro
115       120       125

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
130       135       140

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Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
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 Ala Pro Asp Asn Arg Pro Ala Leu Gly Ser Thr Ala Pro Pro Val His
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 Asn Val Thr Ser Ala Ser Gly Ser Ala Ser Gly Ser Ala Ser Thr Leu
 180 185 190
 Val His Asn Gly Thr Ser Ala Arg Ala Thr Thr Thr Pro Ala Ser Lys
 195 200 205
 Ser Thr Pro Phe Ser Ile Pro Ser His His Ser Asp Thr Pro Thr Thr
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 Leu Ala Ser His Ser Thr Lys Thr Asp Ala Ser Ser Thr His His Ser
 225 230 235 240
 Thr Val Pro Pro Leu Thr Ser Ser Asn His Ser Thr Ser Pro Gln Leu
 245 250 255
 Ser Thr Gly Val Ser Phe Phe Phe Leu Ser Phe His Ile Ser Asn Leu
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 Gln Phe Asn Ser Ser Leu Glu Asp Pro Ser Thr Asp Tyr Tyr Gln Glu
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 Leu Gln Arg Asp Ile Ser Glu Met Phe Leu Gln Ile Tyr Lys Gln Gly
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 Gly Phe Leu Gly Leu Ser Asn Ile Lys Phe Arg Pro Gly Ser Val Val
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 325 330 335
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Asn Pro Ala Val Ala Ala Thr Ser Ala Asn Leu
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MUC-1 DNA SEQ ID NO:23

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2. 3' end of MUC1 gene (contains exon 7, polyA signal and flanking region)

: (SEQ ID NO:24)

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cagctgggtc	cccaaggag	acttggcaca	ttggcatggg	tgtgggacag	gtaaagcatg	1500
caagagggag	aagagggaca	taaggggcat	gcggctgcgg	ggtgttggga	cccaaataaa	1560
taaagcagga	tgacagggtc	cccttcccct	accagggaat	gcctgacagc	gtccagcccc	1620
aaagcctgcc	tgtcccaagg	ctgttgttca	gcacaaacag	gggagggagc	ttggcagggc	1680
aagggcagag	ctggagatca	tgcctcagtg	tccaggtgcc	ctccctccca	atcagcctgg	1740
gggggacagg	acagagattg	agaagggggg	ctctccatgg	cttgggttac	attccaaagg	1800
cagatcatag	ggcagactca	ctgggggtgg	ggggc			1835

3. 5' end of MUC1 gene (contains promoter and first ATG)
: (SEQ ID NO:25)

First ATG is shown as last three residues below:

```

gaattcagaa ttttagaccc tttggccttg ggggccatcc tggagaccct gaggtctaag 60
ctacagcccc tcagccaacc acagaccctt ctctggctcc caaaaggagt tcagtcccag 120
agggtgggtca cccacccttc agggatgaga agttttcaag ggggtattact caggcactaa 180
ccccaggaaa gatgacagca cattgccata aagttttggt tgttttctaa gccagtgcaa 240
ctgcttatttt tagggatttt ccgggatagg gtggggaagt ggaaggaatc ggcgagtaga 300
agagaaagcc tgggaggggtg gaagttaggg atctagggga agtttggtctg atttggggat 360
gcgggtggggg gaggtgctgg atggagttaa gtgaaggata ggggtgcctga gggaggatgc 420
ccgaagtccct cccagaccca ctactcacg gtggcagcgg cgacactcca gtctatcaaa 480
gatccgcggc gatggagagc caggagggcg gggctgcccc tgaggtagcg gggaggccgg 540
ggggccggggg ggcggacggg acgagtgcga tattggcggg ggaaaaaaca aactgcacc 600
gcgtcccgctc cctcccgccc gcccgggccc ggatcccgtc cccacccgcc tgaagccggc 660
ccgacccgga acccgggccg ctggggagtt gggttcacct tggaggccag agagacttgg 720
cgcccggaaag caaagggaat ggcaaggggg aggggggagg gagaacggga gtttgccggag 780
tcagaaggcc cgctttccga cgccggggcg ttgcgcgcgc ttgctcttta agtactcaga 840
ctgcgcggcg cgagccgtcc gcatggtgac gcgtgtoccc gcaaccgaac tgaatggctg 900
ttgtctggca atgcggggag ttgaggtttg gggccgcccc cctagctact cgtgttttct 960
ccggcctgcg agttgggggg ctcccgcttc cccggcccgc tccgtgggcgc gctgacgtca 1020
gatgtcccca ccccgcccag cgctgcccc aagggtctcg ccgcacacaa agctcgccct 1080
cgggcgccgg cgcgcgggcg agagcgggtg tctctcgctt gctgatctga tgcgtccaa 1140
tcccgtgctt cgccgaagtg tttttaaagt gttctttcca acctgtgtct ttggggctga 1200
gaactgtttt ctgaatacag gcggaactgc ttccgtcggc ctgagggcac gctgcgactg 1260
cgggacccaa gttccacgtg ctgcgcggcg ctgggatagc ttccctccct cgtgcctcgc 1320
tgccgcacac acctcttggc tgtcgcgcac tacgcacctc acgtgtgctt ttgccccccg 1380
ctacgtgctt acctgtcccc aataccactc tgcctcccaa aggatagttc tgtgtccgta 1440
aatcccatte tgtcacccca cctactctct gccccccctt tttttgtttt gagacggagc 1500
tttgcctctgt cgcccaggct ggagtgcgat ggcgcgatct cggctcactg caacctccgc 1560
ctcccggggt caagegatte tccgtccca gccctcctgag tagctggggg tacagegccc 1620
gccaccacgc tcggctaatt tttgtagttt ttagtagaga cgagggtttc ccactctggc 1680
caggctgggt ttgaaccctt gacctgtgta tccactcgcc tcggccttcc aaagtgttgg 1740
gattacgggc gtgacgaccg tgccacgcat ctgcctotta agtacataac ggcccacaca 1800
gaacgtgtcc aactcccccg cccacgttcc aacgtccctt cccacatacc tcgggtgcccc 1860
ttccacatac ctacaggacc caccgctta gctccatttc ctccagacgc caccaccacg 1920
cgtcccggag tgccccctcc taaagctccc agccgtccac catgctgtgc gttccctccct 1980
ccctggccac ggcagtgacc ctctctctcc gggccctgct tccctctcgc gggctctgct 2040
gcctcactta ggcagcgtg cccttacttc tctccgcccg gtccgagcgg cccctcagct 2100
tcgggcacca ccccgcgaag gctcccgggt accactagag ggcgggagga gctcctggcc 2160
agtgggtggag agtggcaagg aaggacccta ggggttcacg gagcccaggt ttactccctt 2220
aagtggaaat ttcttcccc actcctcctt ggctttctcc aaggaggga cccaggctgc 2280
tggaaggtcc ggctggggcg gggactgtgg gtccagggga gaacgggggt tggaacggga 2340
caggagcggg ttagaagggt ggggctatcc cgggaagtgg tggggggagg gagcccaaaa 2400
ctagcaccta gtccactcat tatccagccc tcttatttct cggccgctct gcttcagtgg 2460
accgggggag ggcggggaag tggagtggga gccctagggg tgggcttccc gaccttgctg 2520
tacaggacct cgacctagct ggctttgttc cccatcccca cgttagttgt tgccctgagg 2580
ctaaaactag agcccagggg ccccaagttc cagactgccc ctccccctc ccccgagcc 2640
agggagtggg tggtgaaagg gggaggccag ctggagaaca aacgggtagt caggggggtg 2700
agcgattaga gcccttgtag cctaccaggg aatgggtggg gaggaggagg aagaggtagg 2760
aggtagggga gggggcgggg ttttgtcacc tgtcacctgc tcgctgtgcc tagggcgggc 2820
gggcggggag tggggggacc ggtataaagc ggtaggcgcc tgtgcccgtc ccacctctca 2880
agcagccagc gctgcctga atctgttctg cccctcccc acccatttca ccaccaccat 2940
g

```

4. Differentially spliced forms of MUC1

[illegible]

305

: (SEQ ID NO:27)

```

gagctcctgg ccagtgggtgg agagtggcaa ggaaggaccc tagggttcat cggagcccag 60
gtttactccc ttaagtggaa atttcttccc ccactcccct ccttggtttt ctccaaggag 120
ggaaccccag gctgctggaa agtccggctg gggcggggac tgtgggtttc agggtagaac 180
tgcggtgtgga acgggacagg gagcggttag aaggggtggg ctattccggg aagtgggtgt 240
ggggggaggg agcccaaac tagcacctag tccactcatt atccagccct cttattttct 300
ggcgcctct gcttcagtgg acccggggag ggcggggaag tggagtggga gacctagggg 360
tgggtctccc gaccttgctg tacaggacct cgacctagct ggctttgttc cccatcccca 420
gttagttgtt gccctgaggc taaaactaga gcccaggggc cccaagttec agactgcccc 480
tccccctcc cccggagcca gggagtgggt ggtgaaaggg ggaggccagc tggagaagaa 540
acgggtagtc aggggttgca gcattagagc ccttgtagcc ctagccagg aatgggtgga 600
gagagaagag tagagtaggg aggggggttt gtcacctgtc acctgctcgg ctgtgcctag 660
ggcgggaggg ggggagtggg gggaccggtg taaagcggta ggcgcctgtg cccgctccac 720
ctctcaagca gccagcgcc ctctgaatct gttctgcccc ctccccaccc atttcaccac 780
caccatgaca cggggcacc agtctccttt ctctctgctg ctgctcctca cagtgtttac 840
aggtgagggg cagcaggtgg ggagtgggct gccctgttta ggtgggtctt gtgggtcttt 900
tgtgggtttt gctccctggc agatggcacc gtaagttaag gtaagaattg cagacagagg 960
ctgccctgtc tgtgccagaa ggagggagag gctaaggaca ggctgagaag agttgcccc 1020
aaccctgaga gtgggtacca ggggcaagca aatgtcctgt agagaagtct agggggaaga 1080
gagtagggag agggaaggct taagagggga agaatgcag gggccatgag ccaaggccta 1140
tgggcagaga gaaggaggct gctgcaggaa ggaggcggcc aaccagggg ttactgaggc 1200
tgccactcc ccagtcctcc tggattatt tctctggtgg ccaggcttat attttcttct 1260
tgctcttatt tttccttcat aaagacccaa ccctatgact ttaacttctt acagctacca 1320
gagacccctg gcccgcaaca gttgttacag gttctggtca tgcaagctct acccagggtg 1380
gagaaaagga gacttcggct acccagagaa gttcagtgc cagctctact gagaagaatg 1440
ctgtgagtat gaccagcagc gtactctcca gccacagccc cgggttcaggc tctccacca 1500
ctcagggaca ggatgtcact ctggccccgg ccacggaaac agcttcaggc tcagctgcca 1560
cctggggaca ggatgtcacc tcggctccag tcaccaggcc agccctgggc tccaccaccc 1620
cgccagccca cgatgtcacc tcagccccgg acaacaagcc agccccgggc tccaccgccc 1680
ccccagccca ggggtgtcacc tcggccccgg agaccaggcc gccccggggc tccaccgccc 1740
ccccagccca tgggtgtcacc tcggcgccgg acaacaggcc cgccctggcg tccaccgccc 1800
ctccagtcga caatgtcacc tcggcctcag gctctgcac aggtcagct tctactctgg 1860
tgcacaacgg cacctctgcc agggctacca caaccccagc cagcaagagc actccattct 1920
caattcccag ccaccactct gatactccta ccacccttgc cagccatagc accaagactg 1980
atgccagtag cactcaccat agcacggtag ctctctcac ctctccaat cacagcactt 2040
ctccccagtt gtctactggg gtctctttct ttttctgtc ttttcacatt tcaaacctcc 2100
agtttaattc ctctctggaa gatcccagca ccgactacta ccaagagctg cagagagaca 2160
tttctgaat ggtgagtac ggcctttcct tcccctatgt cccctgaagc agccatcaga 2220
actgtccaca ccctttgcat caagcctgag tcccttccct ctaccccag tttttgcaga 2280
tttataaaca aggggggttt ctgggcctct ccaatattaa gttcaggtac agttctgggt 2340
gtggacccag tgtggtggtt ggagggggtg gtggtggtca tgagccgtag ggagggactg 2400
gtgcacttaa ggttggggga agagtgtgta gccagagctg ggaccggtg ctgaagtgcc 2460
catttccctg tgaccaggcc aggatctgtg gtggtacaat tgactctggc cttccgagaa 2520
ggtaccatca atgtccacga cgtggagaca cagttcaatc agtataaac ggaagcagcc 2580
tctcgatata acctgacgat ctcaagacgt cagcgggtgag gctacttccc tgctgcagcc 2640
agcaccatgc cggggccccc ctcttccag tgtctgggtc cccgctcttt ccttagtget 2700
ggcagcggga ggggcgcctc ctctgggaga ctgcctgac cactgctttt ccttttagtg 2760
agtgatgtgc catttccctt ctctgaccag tctggggctg ggggtgccag ctggggcatc 2820
gcgctgctgg tgctggtctg tgttctggtt gcgctggcca ttgtctatct cattgccttg 2880
gtgagtgcag tccctggccc tgatcagagc cccccggtag aaggcactcc atggcctgccc 2940
ataacctcct gctgtctgtc agtgcgcgg aaagaactac gggcagctgg 3000
acatctttcc agcccgggat acctaccatc ctatgagcga gtacccacc taccacaccc 3060
atgggcgcta tgtgcccta gcagtaccga tcgtagcccc tatgagaagg tgagattggg 3120
ccccacaggc aggggaagca gaggggttgg ctgggcaagg attctgaagg ggggtacttg 3180
aaaacccaaa gagcttggaa gaggtgagaa gtggcgtgaa gtgagcaggg gagggctggc 3240
aaggatgagg ggcagaggtc agaggagttt tgggggacag gcctgggagg agactatgga 3300

```


agaaaggggc ccctcaaaag ggagtgcgcc actgccagaa ttc

3343

b. DNA sequence of MUC1Y: (SEQ ID NO:28)

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1 5 10 15
Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
20 25 30
Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
35 40 45
Thr Glu Lys Asn Ala Phe Asn Ser Ser Leu Glu Asp Pro Ser Thr Asp
50 55 60
Tyr Tyr Gln Glu Leu Gln Arg Asp Ile Ser Glu Met Phe Leu Gln Ile
65 70 75 80
Tyr Lys Gln Gly Gly Phe Leu Gly Leu Ser Asn Ile Lys Phe Arg Pro
85 90 95
Gly Ser Val Val Val Gln Leu Thr Leu Ala Phe Arg Glu Gly Thr Ile
100 105 110
Asn Val His Asp Val Glu Thr Gln Phe Asn Gln Tyr Lys Thr Glu Ala
115 120 125
Ala Ser Arg Tyr Asn Leu Thr Ile Ser Asp Val Ser Val Ser Asp Val
130 135 140
Pro Phe Pro Phe Ser Ala Gln Ser Gly Ala Gly Val Pro Gly Trp Gly
145 150 155 160
Ile Ala Leu Leu Val Leu Val Cys Val Leu Val Ala Leu Ala Ile Val
165 170 175
Tyr Leu Ile Ala Leu Ala Val Cys Gln Cys Arg Arg Lys Asn Tyr Gly
180 185 190
Gln Leu Asp Ile Phe Pro Ala Arg Asp Thr Tyr His Pro Met Ser Glu
195 200 205
Tyr Pro Thr Tyr His Thr His Gly Arg Tyr Val Pro Pro Ser Ser Thr
210 215 220
Asp Arg Ser Pro Tyr Glu Lys Val Ser Ala Gly Asn Gly Gly Ser Ser
225 230 235 240
Leu Ser Tyr Thr Asn Pro Ala Val Ala Ala Thr Ser Ala Asn Leu
245 250 255

: (SEQ ID NO:29)

atgacaccgg gcaccagtc tcctttcttc ctgctgctgc tcctcacagt gcttacagtt 60
gttacaggtt ctggtcacgc aagctctacc ccaggtggag aaaaggagac ttcggctacc 120
cagagaagtt cagtgccag ctctactgag aagaatgctt ttaattcctc tctggaagat 180
cccagcaccg actactacca agagctgcag agagacattt ctgaaatgtt tttgcagatt 240

```

tataaacaag ggggttttct gggcctctcc aatattaagt tcaggccagg atctgtggtg 300
gtacaattga ctctggcctt ccgagaaggt accatcaatg tccacgacgt ggagacacag 360
ttcaatcagt ataaaacgga agcagcctct cgatataacc tgacgatctc agacgtcagc 420
gtgagtgatg tgccatttcc tttctctgcc cagtctgggg ctgggggtgcc aggctggggc 480
atcgcgctgc tgggtgctgg ctgtgttctg gttgcgctgg ccattgtcta tctcattgcc 540
ttggctgtct gtcagtgcgc ccgaaagaac tacgggcagc tggacatctt tccagcccgg 600
gataacctacc atcctatgag cgagtacccc acctaccaca cccatggggc ctatgtgccc 660
cctagcagta ccgatcgtag cccctatgag aagggttctg caggtaatgg tggcagcagc 720
ctctcttaca caaaccagc agtggcagcc acttctgcc aattgtag 768

```

c. MUC-1 AA:: (SEQ ID NO:30)

```

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1          5          10          15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
20          25          30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
35          40          45

Thr Glu Lys Asn Ala Leu Ser Thr Gly Val Ser Phe Phe Phe Leu Ser
50          55          60

Phe His Ile Ser Asn Leu Gln Phe Asn Ser Ser Leu Glu Asp Pro Ser
65          70          75          80

Thr Asp Tyr Tyr Gln Glu Leu Gln Arg Asp Ile Ser Glu Met Ala Val
85          90          95

Cys Gln Cys Arg Arg Lys Asn Tyr Gly Leu Leu Asp Ile Phe Pro Ala
100         105         110

Arg Asp Thr Tyr His Pro Met Ser Glu Tyr Pro Thr Tyr His Thr His
115         120         125

Gly Arg Tyr Val Pro Pro Ser Ser Thr Asp Arg Ser Pro Tyr Glu Lys
130         135         140

Val Ser Ala Gly Asn Gly Gly Ser Ser Leu Ser Tyr Thr Asn Pro Ala
145         150         155         160

Val Ala Ala Thr Ser Ala Asn Leu
165

```

: (SEQ ID NO:31)

```

ctccccacc atttaccac caccatgaca ccgggcaccc agtctccttt ctctctgctg 60
ctgctcctca cagtgtttac agttgttaca ggttctggtc atgcaagctc taccccaggt 120
ggagaaaagg agacttcggc taccagaga agttcagtgc ccagctctac tgagaagaat 180
gctttgtcta ctgggggtctc tttctttttc ctgtcttttc acatttcaaa cctccagttt 240
aattcctctc tgggaagatcc cagcaccgac tactaccaag agctgcagag agacatttct 300
gaaatggctg tctgtcagtg ccgccgaaag aactacgggc tgctggacat ctttccagcc 360
cgggatacct accatcctat gagcgagtac cccacctacc acacccatgg gcgctatgtg 420
cccctagca gtaccgatcg tagccctat gagaagggtt ctgcaggtaa tgggtggcagc 480
agcctctctt acacaaaacc agcagtggca gccacttctg ccaacttgta ggggcacgtc 540
gcc 543

```

d. cDNA of a variant of "MUC1Y": (SEQ ID NO:32)

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Leu Thr
 1 5 10 15
 Val Leu Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly Gly Glu Lys
 20 25 30
 Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser Thr Glu Lys
 35 40 45
 Asn Ala Phe Asn Ser Ser Leu Glu Asp Pro Ser Thr Asp Tyr Tyr Gln
 50 55 60
 Glu Leu Gln Arg Asp Ile Ser Glu Met Phe Leu Gln Ile Tyr Lys Gln
 65 70 75 80
 Gly Gly Phe Leu Gly Leu Ser Asn Ile Lys Phe Arg Pro Gly Ser Val
 85 90 95
 Val Val Gln Leu Thr Leu Ala Phe Arg Glu Gly Thr Ile Asn Val His
 100 105 110
 Asp Met Glu Thr Gln Phe Asn Gln Tyr Lys Thr Glu Ala Ala Ser Arg
 115 120 125
 Tyr Asn Leu Thr Ile Ser Asp Val Ser Val Ser Asp Val Pro Phe Pro
 130 135 140
 Phe Ser Ala Gln Ser Gly Ala Gly Val Pro Gly Trp Gly Ile Ala Leu
 145 150 155 160
 Leu Val Leu Val Cys Val Leu Val Ala Leu Ala Ile Val Tyr Leu Ile
 165 170 175
 Ala Leu Ala Val Cys Gln Cys Arg Arg Lys Asn Tyr Gly Gln Leu Asp
 180 185 190
 Ile Phe Pro Ala Arg Asp Thr Tyr His Pro Met Ser Glu Tyr Pro Thr
 195 200 205
 Tyr His Thr His Gly Arg Tyr Val Pro Pro Ser Ser Thr Asp Arg Ser
 210 215 220
 Pro Tyr Glu Lys Val Ser Ala Gly Asn Gly Gly Ser Ser Leu Ser Tyr
 225 230 235 240
 Thr Asn Pro Ala Val Ala Ala Thr Ser Ala Asn Leu
 245 250

: (SEQ ID NO:33)

atgacaccgg gcacccagtc tcctttcttc ctgctgctgc tcctcacagt gcttacaggt 60
 tctgggtcatg caagctctac cccaggtgga gaaaaggaga cttcgggtac ccagagaagt 120
 tcagtgccca gctctactga gaagaatgct tttaattcct ctctggaaga tcccagcacc 180
 gactactacc aagagctgca gagagacatt tctgaaatgt ttttgcatat ttataaaca 240
 ggggggttttc tgggcctctc caatattaag ttcaggccag gatctgtggt ggtacaattg 300
 actctggcct tccgagaagg taccatcaat gtccacgaca tggagacaca gttcaatcag 360
 tataaaacgg aagcagcctc tcgatataac ctgacgatct cagacgtcag cgtgagtgat 420
 gtgccatttc ctttctctgc ccagtcctggg gctgggggtgc caggctgggg catcgcgctg 480
 ctgggtgctgg tctgtgttct gggtgcgctg gccattgtct atctcattgc cttggctgtc 540

```

tgtcagtgcc gccgaaagaa ctacgggacag ctggacatct ttccagcccg ggatacctac 600
catcctatga gcgagtaccc cacctaccac acccatgggc gctatgtgcc ccttagcagt 660
accgatcgta gcccctatga gaaggtttct gcaggtaatg gtggcagcag cctctcttac 720
acaaaccag cagtggcagc cacttctgcc aactttag 759

```

Reference: no published reference, only the database information

e. MUC1X or MUC1Z partial cDNA sequence: : (SEQ ID NO:34)

```

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1           5           10           15
Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
20           25           30
Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
35           40           45
Thr Glu Lys Asn Ala Leu Ser Thr Gly Val Ser Phe Phe Phe Leu Ser
50           55           60
Phe His Ile Ser Asn Leu Gln Phe Asn Ser Ser Leu Glu
65           70           75

```

f. S81781, cDNA: (SEQ ID NO:35)

```

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1           5           10           15
Val Leu Thr Ala Thr Thr Ala Pro Lys Pro Ala Thr Val Val Thr Gly
20           25           30
Ser Gly His Ala Ser Ser Thr Pro Gly Gly Glu Lys Glu Thr Ser Ala
35           40           45
Thr Gln Arg Ser Ser Val Pro Ser Ser Thr Glu Lys Asn Ala Val Ser
50           55           60
Met Thr Ser Ser Val Leu Ser Ser His Ser Pro Gly Ser Gly Ser Ser
65           70           75           80
Thr Thr Gln Gly Gln Asp Val Thr Leu Ala Pro Ala Thr Glu Pro Ala
85           90           95
Ser Gly Ser Ala Ala Thr Trp Gly Gln Asp Val Thr Ser
100          105

```

: (SEQ ID NO:36)

```

accaccacca tgacaccggg caccagctct cctttcttcc tgctgctgct cctcacagtg 60
cttacagcta ccacagcccc taaaccgcga acagttgtta caggttctgg tcatgcaagc 120
tctaccccag gtggagaaaa ggagacttcg gctaccaga gaagttcagt gccagctct 180
actgagaaga atgctgtgag tatgaccagc agcgtactct ccagccacag ccccggttca 240
ggctcctcca ccaactcagg acaggatgtc actctgcccc cgccacgga accagcttca 300
ggttcagctg ccacctgggg acaggatgtc acctcg 336

```

Reference: Int. J. Cancer 66 (1), 55-59 (1996)

g. M32738, partial cDNA of MUC1 splice variant A: : (SEQ ID NO:37)

```

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1           5           10           15

Val Leu Thr Ala Thr Thr Ala Pro Lys Pro Ala Thr Val Val Thr Gly
          20           25           30

Ser Gly His Ala Ser Ser Thr Pro Gly Gly Glu Lys Glu Thr Ser Ala
          35           40           45

Thr Gln Arg Ser Ser Val Pro Ser Ser Thr Glu Lys Asn Ala Val Ser
          50           55           60

Met Thr Ser Ser Val Leu Ser Ser His Ser Pro Gly Ser Gly Ser Ser
65           70           75           80

Thr Thr Gln Gly Gln Asp Val Thr Leu Ala Pro Ala Thr Glu Pro Ala
          85           90           95

Ser Gly Ser Ala Ala Thr Trp Gly Gln Asp Val Thr Ser Val Pro Val
          100          105          110

Thr Arg Pro Ala Leu Gly Ser Thr Thr Pro Pro Ala His Asp Val Thr
          115          120          125

Ser Ala Pro Asp Asn Lys Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala
          130          135          140

His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala
145           150           155

```

: (SEQ ID NO:38)

```

gcgcctgcct gaatctgttc tgccccctcc ccaccattt caccaccacc atgacaccgg 60
gcacccagtc tcctttcttc ctgctgctgc tctcacagt gcttacagct accacagccc 120
ctaaacccgc aacagttggt acaggttctg gtcattgcaag ctctacccca ggtggagaaa 180
aggagacttc ggctacccag agaagttcag tgcccagctc tactgagaag aatgctgtga 240
gtatgaccag cagcgtaact tccagccaca gcccgggttc aggtctctcc accactcagg 300
gacaggatgt cactctggcc cgggccacgg aaccagcttc aggttcagct gccacctggg 360
gacaggatgt cacctcgggc ccagtcacca ggccagccct gggctccacc accccgccag 420
cccacgatgt cacctcagcc ccggacaaca agccagcccc gggctccacc gcccccccag 480
cccacgggtgt cacctcgggc ccggacacca ggccggcc 518

```

Reference: J. Biol. Chem. 265, 5573-5578 (1990)

h. Z17324, partial cDNA of MUC1 splice variant C: (SEQ ID NO:39)

```

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1           5           10           15

Val Leu Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly Gly Glu Lys
          20           25           30

Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro
          35           40

```

: (SEQ ID NO:40)

```

ccgctccacc tctcaagcag ccagcgctg cctgaatctg ttctgcccc tccccaccca 60
tttcaccacc accatgacac cgggcaccca gtctcctttc ttctgctgc tgctcctcac 120
agtgtttaca ggttctggtc atgcaagctc taccacaggt ggagaaaagg agattcggc 180
taccagaga agttcagtgc ccag                                     204

```

Reference: no literature reference, a direct submission to the database

i. Z17325, partial cDNA of MUC1 splice variant D

: (SEQ ID NO:41)

```

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Thr
1           5           10           15
Val Leu Thr Gly Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser
          20           25           30

```

Val Pro

: (SEQ ID NO:42)

```

ccgctccacc tctcaagcag ccagcgctg cctgaatctg ttctgcccc tccccaccca 60
tttcaccacc accatgacac cgggcaccca gtctcctttc ttctgctgc tgctcctcac 120
agtgtttaca ggtggagaaa aggagacttc ggctaccacag agaagttcag tgcccag 177

```

5. CTL epitopes of MUC1: : (SEQ ID NO:43)

```

Ser Thr Ala Pro Pro Val His Asn Val
1           5

```

Reference: Blood 93:4309-4317, 1999

: (SEQ ID NO:44)

```

Leu Leu Leu Leu Thr Val Leu Thr Val
1           5

```

Reference: Blood 93:4309-4317, 1999

: (SEQ ID NO:45)

```

Ser Thr Ala Pro Pro Ala His Gly Val
1           5

```

Reference J Immunology 155:4766-4774, 1995; J Immunology 159:5211-5218, 1997

: (SEQ ID NO:46)

```

Ala Pro Asp Thr Arg Pro Ala
1           5

```

Reference J Immunology 159:5211-5218, 1997

6. CD4 T helper epitopes of MUC1

: (SEQ ID NO:47)

Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr

1

5

10

for HLA DR3 Reference: Cancer Research 58: 5066-5070, 1998

IV. Sequences for DNA vaccine vectors:

1. HCMV promoter/enhancer; K01484 Mark Stinski U Iowa

490 bp of promoter sequence, to transcriptional start

: (SEQ ID NO:48)

```

ggcgaccgcc cagcgacccc cgcccgttga cgtcaatagt gacgtatggt cccatagtaa 60
cgccaatagg gactttccat tgacgtcaat ggggtggagta ttacggtaa actgccact 120
tggcagtaca tcaagtgtat catatgccaa gtccgcccc tattgacgtc aatgacggta 180
aatggccgcg ctagcattat gccagttaca tgaccttac ggagtttcc acttggcagt 240
acatctacgt attagtcac gctattacca tgggtgatgcg gttttggcag tacaccaatg 300
ggcgtggata gcggtttgac tcacggggat ttccaagtct ccacccatt gacgtcaatg 360
ggagtttggt ttggcaccaa aatcaacggg actttccaaa atgtcgtaat aaccccgccc 420
cgttgacgca aatggcggtt aggcgtgtac ggtgggagggt ctatatagca gagctcgttt 480
agtgaaccgt cagatcgctt ggagacgcca tccacgtgt tttgacctcc atagaagaca 540
ccgggaccga tccagcctcc gggcgccgga acggtgcatt ggaacgcgga ttccccgtgc 600
caagagtgc gtaagt                                     616

```

Reference: J. Virol. 49, 190-199 (1984); Proc. Natl. Acad. Sci. U.S.A. 81, 659-663 (1984)

2. HCMV promoter/enhancer; K03104

: (SEQ ID NO:49)

737bp of promoter sequence, to +193bp; includes exon 1 and part of intron A

```

aatcaatatt ggccattagc catattatc attgggtata tagcataaat caatattggc 60
tattggccat tgcatacgtt gtatccatat cataatatgt acatttatat tggctcatgt 120
ccaacattac cgccatgttg acattgatta ttgactagtt attaatagta atcaattacg 180
gggtcattag ttcataagccc atatatggag ttccgcgtta cataacttac ggtaaatggc 240
ccgcctggct gaccgcccac cgacccccgc ccattgacgt caataatgac gtatgttccc 300
atagtaacgc caatagggac ttccattga cgtcaatggg tggagtattt acggtaaact 360
gcccacttgg cagtacatca agtgtatcat atgccaagta cgcccctat tgacgtcaat 420
gacggtaaat ggcccgcctg gcattatgcc cagtacatga ccttatggga ctttccact 480
tggcagtaca tctacgtatt agtcacgtc attaccatgg tgatgcggtt ttggcagtac 540
atcaatgggc gtggatagcg gtttgactca cggggatttc caagtctcca cccattgac 600
gtcaatggga gtttggtttg gcacaaaat caacgggact ttccaaaatg tcgtaacaac 660
tccgccccat tgacgcaaat gggcggtagg cgtgtacggt gggaggtcta tataagcaga 720
gctcgtttag tgaaccgtca gatcgctgg agacgccatc cacgctggtt tgacctccat 780
agaagacacc gggaccgatc cagcctccgc ggccgggaac ggtgcattgg aacgcggatt 840
ccccgtgcca agagtgcgt aagtaaccgc tatagagtct ataggccac ccccttggt 900
tcttatgcat gctatactgt ttttggttgc                                     930

```

Reference: Cell 41:521-530, 1985

3. HCMV promoter, exon 1, intron A and part of exon 2; M60321

: (SEQ ID NO:50)

```

ctgcagtga taataaaatg tgtgtttgtc cgaaatacgc gttttgagat ttctgtcgcc 60
gactaaattc atgtcgcgcg atagtgggtt ttatcgccga tagagatggc gatattggaa 120
aaatcgatat ttgaaaatat ggcattatga aaatgtcgcc gatgtgagtt tctgtgtaac 180

```

```

tgatatacgcc atttttccaa aagtgatttt tgggcatacg cgatatctgg cgatacggct 240
tatatacgttt acgggggatg gcgatagacg acttttggcga cttgggcgat tctgtgtgtc 300
gcaaatatcg cagtttcgat ataggtgaca gacgatatga ggctatatcg ccgatagagg 360
cgacatcaag ctggcacatg gccaatgcat atcgatctat acattgaatc aatattggca 420
attagccata ttagtcattg gttatatagc ataaatcaat attggctatt ggccattgca 480
tacgttgtat ctatatcata atatgtacat ttatatggc tcatgtccaa tatgaccgcc 540
atgttgacat tgattattga ctagttatta atagtaatca attacggggc cattagttca 600
tagcccatat atggagttcc gcgttacata acttacggta aatggcccgc ctogtgaccg 660
cccaacgacc ccgcccatt gacgtcaata atgacgtatg tcccatagt aacgccata 720
gggactttcc attgacgtca atgggtggag tatttacggg aaactgccc cttggcagta 780
catcaagtgt atcatatgcc aagtcgggcc cctattgac gtcaatgacg gtaaattggcc 840
cgcttgccat tatgcccagt acatgacctt acgggacttt cctacttggc agtacatcta 900
cgtattagtc atcgctatta ccatggtgat gcggttttgg cagtacacca atgggcgtgg 960
ataggtgatt gactcacggg gatttccaag tctccacccc attgacgtca attgggagttt 1020
gttttggcac caaaatcaac gggactttcc aaaatgtcgt aataaccccg cccggtgac 1080
gcaaatgggc ggtaggcgtg tacggtggga ggtctatata agcagagctc gtttagtgaa 1140
ccgtcagatc gcctggagac gccatccacg ctgttttgac ctccatagaa gacaccggga 1200
ccgatccagc ctccgcggcc gggaacggtg cattggaacg cggattcccc gtgccaagag 1260
tgacgtaagt accgcctata gactctatag gcacacccct ttggtcttta tgcattgctat 1320
actgtttttg gcttggggcc tatacacccc cgctccttat gctatagggt atggtatagc 1380
ttagcctata ggtgtgggtt attgaccatt attgaccact cccctattgg tgacgatact 1440
ttccattact aatccataac atggctcttt gccacaacta tctctattgg ctatatgcca 1500
atactctgtc cttcagagac tgacacggac tctgtatttt tacaggatgg ggtcccat 1560
attattttaca aattcacata tacaacaacg ccgtcccccg tgcccgagc ttttattaaa 1620
catagcgtgg gatctccacg cgaatctcgg gtacgtgttc cggacatggg ctcttctccg 1680
gtagcggcgg agcttccaca tccgagccct ggtcccatgc ctccagcggc tcatggtcgc 1740
tcggcagctc cttgtccta acagtggagg ccagacttag gcacagcaca atgccacca 1800
ccaccatgtg gccgcacaag gccgtggcgg taggggtatgt gtctgaaaat agctcggag 1860
attgggctcg caccgtgacg cagatggaa gacttaaggca gggcgagaag aagatgcagg 1920
cagctgagtt gttgtattct gataagagtc agaggtaact cccgttgccg tgctgttaac 1980
gggtggagggc agtgtagtct gagcagtact cgttgctgcc gcgcgcgcca ccagacataa 2040
tagctgacag actaacagac tgttcctttc catgggtctt ttctgcagtc accgtccttg 2100
acacgatgga gtccctctgcc aagagaaaaga tggaccctga taatcctgac gagggccctt 2160
cttccaaggt gccacgggtac gtgtcggggg ttgtgcccc cctttttttt ataaaattgt 2220
attaatgtta tatacatatc tctgtatgt gacccatgtg cttatgactc tatttctcat 2280
gtgttttaggc ccgagacacc cgtgaccaag gccacgacgt tcttcagac tatgttgagg 2340
aaggagggtta acagtcagct g

```

Reference: Nucleic Acids Res. 19, 3979-3986 (1991)

4. HCMV promoter/enhancer with upstream NF1 binding sites; includes 1140bp of upstream promoter with 748bp of exon 1 and intron A; X03922

: (SEQ ID NO:51)

```

ctgcagttaa taataaaatg tgtgtttgtc cgaaatacgc gtttgagatt tctgtcccga 60
ctaaattcat gtcgcgcgat agtgggtgtt atcgccgata gagatggcga tattggaaaa 120
atcgatatatt gaaaatatgg catattgaaa atgtcgccga tgtgagtttc tgtgtaactg 180
atatcgccat ttttccaaaa gttgattttt gggcatacgc gatatactggc gatagcgtta 240
tatcgtttac ggggggatggc gatagacgcc tttgggtgact tgggcgattc tgtgtgtcgc 300
aaatatcgca gtttcgatat aggtgacaga cgatatgagg ctatatcgcc gatagaggcg 360
acatcaagct ggcacatggc caatgcatac cgatctatac attgaatcaa tattggccat 420
tagccatatt attcattggt tatatagcat aaatcaatat tggctatttg ccattgcata 480
cgttgatccc atatacata atgtacattt atattggctc atgtccaaca ttaccgccat 540
gttgacattg attattgact agttattaat agtaatcaat tacgggggtca ttagttcata 600
gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct ggtgaccgc 660
ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag 720
ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac 780
atcaagtgtt tcatatgcca agtacgcccc ctattgacgt caatgacggg aaatggcccg 840
cctggcatta tgcccagtag atgaccttat gggactttcc tacttggcag tacatctacg 900

```


tattagtc	cgctattacc	atggtgatgc	ggttttggca	gtacatcaat	gggcgtggat	960
agcggtttga	ctcacgggga	tttccaagtc	tccaccccat	tgacgtcaat	gggagtttgt	1020
tttggcacca	aaatcaacgg	gactttccaa	aatgtcgtaa	caactccgcc	ccattgacgc	1080
aaatggcg	taggcgtgta	cgggtggagg	tctatataag	cagagctcgt	ttagtgaacc	1140
gtcagatcgc	ctggagacgc	catccacgct	gttttgacct	ccatagaaga	caccgggacc	1200
gatccagcct	ccgcggccgg	gaacggtgca	ttggaacgcg	gattccccgt	gccaaagagt	1260
acgtaagtac	cgccatataga	gtctataggg	ccacccccct	ggcttcttat	gcatgctata	1320
ctgttttttg	cttgggggtct	atacaccccc	gcttctctcat	gttatagggtg	atgggtatagc	1380
ttagccata	ggtgtgggtt	attgaccatt	attgaccact	cccctattgg	tgacgatact	1440
ttccattata	aatccataac	atggctcttt	gcacaactct	ctttattggc	tatatgccaa	1500
tacactgtcc	ttcagagact	gacacggact	ctgtattttt	acaggatggg	gtctcattta	1560
ttattttacaa	attcacatat	acaacaccac	cgtccccagt	gcccgcagtt	tttattaaac	1620
ataacgtggg	atctccagcg	aatctcgggt	acgtgttccg	gacatggggc	tcttctcccg	1680
tagcggcgga	gcttctacat	ccagccctgc	tcccatcctc	ccactcatgg	tcctcggcag	1740
ctccttgctc	ctaacagtgg	aggccagact	taggcacagc	acgatgccc	ccaccaccag	1800
tgtgccaca	aggccgtggc	ggtaggggtat	gtgtctgaaa	atgagctc		1848

Reference: EMBO J. 5 (6), 1367-1371 (1986)

5. Various strains of HMCV IE promoter/enhancer; these are different from each other at a few residues compared to the two sequences listed above in 1 and 2; M64940-M64944

M64940

: (SEQ ID NO:52)

ggcacatggc	caatgcatat	cgatatatac	attgaatcaa	tattggctat	tagccatatt	60
agtcattggg	tatatagcat	aaatcaatat	tggctaattg	ccattgcata	cattgcagct	120
atagcataat	atgtacattt	atattggctc	atgtccaata	tgaccgccat	gttgacattg	180
attattgact	agttattaat	agtaatcaat	tacgggtgca	ttagttcata	gcccataatat	240
ggagttcccg	cgttacataa	cttacggtaa	atggccccgc	tggctgaccg	cccaacgacc	300
cccgcccatt	gacgtcaata	atgacgtgag	ttcccatagt	aacgccaata	gggactttcc	360
attgacgtca	atggggaggag	tatttacggg	aaactgcccc	cttggcagta	catcaagtgt	420
atcatatgcc	aagtacgccc	cctattgacg	tcaatgacgg	taaatggccc	gcctggcatt	480
atgcccagta	catgacctta	cgggactttc	ctacttggca	gtacatctac	gtattagtca	540
tcgctattac	catggtgatg	cggttttggc	agtacatcaa	tgggcgtgga	tagcgggttg	600
actcacgggg	atttccaagt	ctccacccca	ttgacgtcaa	tgggagtttg	ttttggcacc	660
aaattcaacg	ggactttcca	aaatgtcgta	ataactccgc	cccattgacg	caaatgggcg	720
gtaggcgtgt	acgatgggtg	gtctatataa	gcagagctcg	tttagtgaac	cgtcagatcg	780
cctggagacg	ccatccacgc	tgttttgacc	tccatagaag	acaccgggac	cgatccagcc	840
tccgcggccg	ggaacggtgc	attggaacgc	ggattc			876

M64941

: (SEQ ID NO:53)

ggcacatggc	caatgcatat	cgatatatac	attgaatcaa	tattggccat	tagccatatt	60
agtcattggg	tatatagcat	aaatcaatat	tggctaattg	ccattgcata	cgttgcatct	120
atatacataat	gtgtacattt	atattggctc	atgtccaata	tgaccgccat	gttgacattg	180
attattgact	agttattaat	agtaatcaat	tacgggtgca	ttagttcata	gcccataatat	240
ggagttccgc	gttacataac	ttacggtaaa	tggccccgct	ggctgaccgc	ccaacgaccc	300
ccgcccattg	acgtcaataa	tgacgtgggt	tcccatagta	acgccaatag	ggactttcca	360
ttgacgtcaa	tgggaggagt	atttacggta	aactgcccac	ttggcagtac	atcaagtgtg	420
tcatatgcca	agtacgcccc	ctattgacgt	caatgacggt	aaatggcccc	cctggcatta	480
tgcccagtag	atgaccttac	gggactttcc	tacttggcag	tacatctacg	tattagtcac	540
cgctattacc	ttggtgatgc	ggttttggca	gtacatcaat	gggcgtggat	agcggtttga	600
ctcacgggga	tttccaagtc	tccaccccat	tgacgtcaat	gggagtttgt	tttggcacca	660
aattcaacgg	gactttccaa	aatgtcgtaa	taactccgcc	ccattgacgc	aaatggggcg	720
taggcgtgta	ctatgggagg	tctatataag	cagagctcgt	ttagtgaacc	gtcagatcgc	780
ctggagacgc	catccacgct	gttttgacct	ccatagaaga	caccgggacc	gatccagcct	840
ccgcggcccg	gaacggtgca	ttggaacgcg	gattc			875

M64942

: (SEQ ID NO:54)

```

ggcacatggc caatgcatat cgatatatac attgaatcaa tattggctat tagccatatt 60
agtcattggg tatatagcat aaatcaatat tggctaattg ccattgcata cattgcagct 120
atagcataat atgtacattt atattggctc atgtccaata tgaccgccat gttgacattg 180
attattgact agttattaat agtaatcaat tacgggggtca ttagttcata gcccatatat 240
ggagttcccg cgttacataa cttacggtaa atggcccggc tggctgaccg cccaacgacc 300
cccgccatt gacgtcaata ttgacgtgag ttcccatagt aacgccaata gggactttcc 360
attgacgtca atgggtggag tatttacggg aaactgcccc cttggcagta catcaagtgt 420
atcatatgcc aagtacggcc cctattgacg tcaatgacgg taaatggccc gcctggcatt 480
atgcccagta catgacctta cgggactttc ctacttggca gtacatctac gtattagtca 540
tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg 600
actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 660
aaattcaacg ggactttcca aaatgtcgta ataactccgc ccattgacg caaatgggag 720
gtaggcgtgt acgattggga ggtctatata agcagagctc gtttagtgaa ccgtcagatc 780
gcctggagac gccatccacg ctgttttgac ctccatagaa gacaccggga ccgatccagc 840
ctccgcgggc gggaacggtg cattggaacg cggatcc 877

```

M64943

: (SEQ ID NO:55)

```

ggcacatggc caatgcatat cgatctatac attgaatcaa tattggccat tagccatatt 60
agtcattggg tatatagcat aaatcaatat tgactatttg ccattgcata cgttgatcc 120
atatcataat atgtacattt atattggctc atgtccaata tgaccgccat gttgacattg 180
attattgact agttattaat agtaatcaat tacagggtca ttagttcata gcccatatat 240
ggagttcccg gttacataac ttacggtaaa tggcccgcct ggctgaccgc ccaacgacc 300
ccgccattg acgtcaataa cgacgtatgt tcccatagta acgctaatag ggactttcca 360
ttgacgtcaa tgggaggagt atttacggta aactgcccac ttggcagtac atcaagtgt 420
tcatatgcc agtacgcccc ccattgacgt caatgacggg aaatggccc cctggcatta 480
tgcccagtac atgaccttac gggactttcc tacttggcag tacatctacg tattagtcac 540
cactattacc atggtgatgc ggttttggca gtacatcaat ggggtgtggat agcggtttga 600
ctcacgggga ttccaagtc tccaccccat tgacgtcaat gggagtttgt tttggcacc 660
aaatcaacgg gactttccaa aatgtcgtaa taactccgcc ccattgacgc aaatgggagg 720
taggcgtgt cagtgggagg tctatataag cagagctcgt ttagtgaacc gtcagatcgc 780
ctggagacgc catccacgct gttttgacct ccatagaaga caccgggacc gatccagcct 840
cccgggcccg gaacggtgca ttggaacgcg gatt 874

```

M64944

: (SEQ ID NO:56)

```

ggcacatggc caatgcatat cgatatatac attgaatcaa tattggccat tagccatatt 60
agtcattggg tatatagcgt aaatcaatat tggctaattg ccattgcata cgttgatct 120
atatcataat gtgtacattt atattggctc atgtccaata tgaccgccat gttgacattg 180
attattgact agttattaat agtaatcaat tacgggggtca ttagttcata gcccatatat 240
ggagttcccg cgttacataa cttacggtaa atggcccggc tggctgaccg cccaacgacc 300
cccgccatt gacgtcaata atgacgtgag ttcccatagt aacgccaata gggactttcc 360
attgacgtca atgggtggag tatttacggg aaactgcccc cttggcagta catcaagtgt 420
atcatatgcc aagtacggcc cctattgacg tcaatgacgg taaatggccc gcctggcatt 480
atgcccagta catgacctta cgggactttc ctacttggca gtacatctac gtattagtca 540
tcgctattac catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg 600
actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 660
aaattcaacg ggactttcca aaatgtcgta ataactccgc ccattgacg caaatgggag 720
gtaggcgtgt actatgggag gtctatataa gcagagctcg ttagtgaac cgatcagatc 780
cctggagacg ccatccacgc tgttttgacc tccatagaag acaccgggac cgatccagc 840
tccgcgcccg ggaacggtgc attggaacgc ggatcc 876

```

Reference: J. Clin. Microbiol. 29, 2494-2502 (1991)

6. SV40 polyadenylation signal (late and early); J02400

: (SEQ ID NO:57)

```

ggggatccag acatgataag atacattgat gagtttggac aaaccacaac tagaatgcag 60
tgaaaaaat gctttatttg tgaaatttgt gatgctattg ctttatttgt aaccattata 120
agctgcaata aacaagttaa caacaacaat tgcattcatt ttatgtttca gggtcagggg 180
gaggtgtggg aggtttttta aagcaagtaa aacctctaca aatgtggtat ggctgattat 240
gatcatgaac                                     250

```

Reference: Proc. Natl. Acad. Sci. U.S.A. 78 (1), 100-104 (1981)

7. Rabbit β globin intron 2; J00600

: (SEQ ID NO:58)

```

ggatcctgag aacttcaggg tgagtttggg gacccttgat tggtctttct ttttcgctat 60
tgtaaaattc atgttatatg gagggggcaa agttttcagg gtgttggtta gaatgggaag 120
atgtcccttg tatcaccatg gaccctcatg ataatttgt ttctttcact ttctactctg 180
ttgacaacca ttgtctcttc ttattttctt ttcatcttct gtaacttttt cgttaaactt 240
tagcttgcat ttgtaacgaa tttttaaatt cacttttgtt tatttgtcag attgtaagta 300
ctttctctaa tcaacttttt ttcaaggcaa tcagggtata ttatattgta cttcagcaca 360
gttttagaga acaattgtta taattaaatg ataaggtaga atattttctgc atataaattc 420
tggtcggcgt ggaaatattc ttattggtag aaacaactac accctggtca tcatcctgcc 480
tttctcttta tggttacaat gatatacact gtttgagatg aggataaaat actctgagtc 540
caaaccgggc cctctgcta accatgttca tgccttcttc tctttctctac agctcctggg 600
caacgtgctg                                     610

```

References: Cell 10, 549-558 (1977); Cell 18, 1285-1297 (1979)

8. Minimal synthetic rabbit β globin polyadenylation signal

: (SEQ ID NO:59)

```

aataaaaagat ccagagctct agagatctgt gtgttggttt tttgtgtg 48

```

Reference: Genes and Development 3: 1019-1025, 1989

V. IL-18 sequences to claim

1. Mature consensus human IL-18 linked to an HC signal sequence, with intron included and underlined. Bold areas are from the HC signal sequence, and the unbolded are the linked mature human IL-18 sequence

: (SEQ ID NO:60)

```

ATGGGGTCAACCGCCATCCTCGGCCTCCTCCTGGCTGTTCTCCAAGGTCAGTCCTGCCGAGGTCTTGAGG
TCACAGAGGAGAACGGGTGGAAAGGAGCCCCCTGATTCAAATTTTGTGTCTCCCCCACAGGAGTCTGTGCC
tacttt ggcaagctt gaatctaaat tatcagtcac aagaaatttg aatgaccaag
ttctcttcat tgaccaagga aatcggcctc tatttgaaga tatgactgat tctgactgta
gagataatgc accccggacc atatttatta taagtatgta taaagatagc cagcctagag
gtatggctgt aactatctct gtgaagtgtg agaaaatttc aactctctcc tgtgagaaca
aaattatttc ctttaaggaa atgaatcctc ctgataacat caaggataca aaaagtgaaca
tcatattctt tcagagaagt gtcccaggac atgataataa gatgcaattt gaatcttcat
catacgaagg atactttcta gcttgtgaaa aagagagaga cttttttaaa ctcatattga
aaaaagagga tgaattgggg gatagatcta taatgttcac tgttcaaaac gaagactag

```

: (SEQ ID NO:61)

```

atgggggtcaa ccgccaatcct cggcctcctc ctggctgttc tccaaggtea gtccctgccga 60
ggctcttgagg tcacagagga gaacgggttg aaaggagccc ctgattcaaa ttttgtgtct 120
ccccacagg agtctgtgcc tactttggca agcttgaatc taaattatca gtcataagaa 180
atttgaatga ccaagttctc ttcattgacc aaggaaatcg gcctctattt gaagatatga 240

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ctgattctga ctgtagagat aatgcacccc ggaccatatt tattataagt atgtataaag 300
atagccagcc tagaggtagt gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc 360
tctcctgtga gaacaaaatt atttccttta aggaaatgaa tctcctgat aacatcaagg 420
atacaaaaag tgacatcata ttctttcaga gaagtgtccc aggacatgat aataagatgc 480
aatttgaatc ttcacatac gaaggatact ttctagcttg tgaaaaagag agagaccttt 540
ttaaactcat tttgaaaaaa gaggatgaat tgggggatag atctataatg ttcactgttc 600
aaaacgaaga ctag 614

```

: (SEQ ID NO:62)

ATGGGGTCAACCGCCATCCTCGGCCTCCTCCTGGCTGTTCTCCAAGGTCAGTCCTGCC
GAGGTCCTTGAGGTCACAGAGGAGAACGGGTGGAAGGAGCCCTGATTCAAATTT
GTGTCCTCCCCACAGGAGTCTGTGCC

```

atgggggtcaa cgcctatcct cggcctcctc ctggctgttc tccaagggtca gtcctgccga 60
ggctcttgagg tcacagagga gaacgggtgg aaaggagccc ctgattcaaa tttgtgtct 120
ccccacagg agtctgtgcc 140

```

: (SEQ ID NO:63)

tacttt ggcaagctt gaatctaat tatcagtc atgaaatttg aatgaccaag ttcttctat tgaccaagga aatggcctc
tattgaaga tatgactgat tctgactgta gagataatgc accccggacc atatttatta taagtatgta taaagatagc cagcctagag
gtatggctgt aactatctct gtgaagtgtg agaaaatttc aactctctc tgtgagaaca aaattatttc ctttaaggaa atgaatcctc
ctgataacat caaggatata aaaagtgaac tcatattctt tcagagaagt gtcccaggac atgataataa gatgcaattt gaattctcat
catagcaagg atactticta gctgtgaaa aagagagaga cctttttaa ctcatttga aaaaagagga tgaattgggg gatagatcta
taattgtcac tgttcaaac gaagactag

```

tactttggca agcttgaatc taaattatca gtcataagaa atttgaatga ccaagttctc 60
ttcattgacc aaggaaatcg gcctctatct gaagatatga ctgattctga ctgtagagat 120
aatgcacccc ggaccatatt tattataagt atgtataaag atagccagcc tagaggtagt 180
gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc tctcctgtga gaacaaaatt 240
atttccttta aggaaatgaa tctcctgat aacatcaagg atacaaaaag tgacatcata 300
ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcacatac 360
gaaggatact ttctagcttg tgaaaaagag agagaccttt ttaaactcat tttgaaaaaa 420
gaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga ctag 474

```

: (SEQ ID NO:64)

MGSTAILGLLLAVLQGVCA

```

Met Gly Ser Thr Ala Ile Leu Gly Leu Leu Leu Ala Val Leu Gln Gly
1           5           10           15

```

Val Cys Ala

: (SEQ ID NO:65)

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEK
ISTLSCENKIIISFKEMNPPDNIDTKSDIIFFQRSVPGHDNKMQFESSYEGYFLACEKERDLFKLILKK
EDELGDRSIMFTVQNE

```

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1           5           10           15

```

```

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20           25           30

```

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile

35	40	45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile		
50	55	60
Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile		
65	70	75 80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys		
85	90	95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys		
100	105	110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu		
115	120	125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu		
130	135	140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp		
145	150	155

2. Mature consensus human IL-18 linked to a human LC signal sequence, with no intron. Bold areas are from the LC signal sequence, and the unbolded are the linked mature human IL-18 sequence.

: (SEQ ID NO:66)

ATGGCCTGGACCGTTCTCCTCCTCGGCCTCCTCTCTCACTGCACAGGCTCTGTGACCTCC tacttt
 ggcaagctt gaatctaaat tatcagtcac aagaaatttg aatgaccaag ttctcttcat
 tgaccaagga aatcggcctc tatttgaaga tatgactgat tctgactgta gagataatgc
 accccggacc atatttatta taagtatgta taaagatagc cagcctagag gtatggctgt
 aactatctct gtgaagtgtg agaaaatttc aactctctcc tgtgagaaca aaattatttc
 ctttaaggaa atgaatcctc ctgataacat caaggatata aaaagtgaca tcatattctt
 tcagagaagt gtcccaggac atgataataa gatgcaattt gaatcttcat catacgaagg
 atactttcta gcttgtgaaa aagagagaga ctttttttaa ctcattttga aaaaagagga
 tgaattgggg gatagatcta taatgttcac tgttcaaac gaagactag

atggcctgga ccgttctcct cctcggcctc ctctctcact gcacaggctc tgtgacctcc 60
 tactttggca agcttgaatc taaattatca gtcataagaa atttgaatga ccaagttctc 120
 ttcatgacc aaggaaatcg gcctctatct gaagatatga ctgattctga ctgtagagat 180
 aatgcacccc ggaccatatt tattataagt atgtataaag atagccagcc tagaggtagt 240
 gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc tctcctgtga gaacaaaatt 300
 atttccttta aggaaatgaa tctcctgat aacatcaagg atacaaaaag tgacatcata 360
 ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcatcatac 420
 gaaggatact ttctagcttg tgaaaaagag agagaccttt ttaaactcat ttgaaaaaaa 480
 gaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga cttag 534

: (SEQ ID NO:67)

ATGGCCTGGACCGTTCTCCTCCTCGGCCTCCTCTCTCACTGCACAGGCTCTGTGACCTCC

atggcctgga ccgttctcct cctcggcctc ctctctcact gcacaggctc tgtgacctcc 60

: (SEQ ID NO:68)

tacttt ggcaagctt gaatctaaat tatcagtcac aagaaatttg aatgaccaag
 ttctcttcat tgaccaagga aatcggcctc tatttgaaga tatgactgat
 tctgactgta gagataatgc accccggacc atatttatta taagtatgta
 taaagatagc cagcctagag gtatggctgt aactatctct gtgaagtgtg

agaaaatttc aactctctcc tgtgagaaca aaattatttc cttaaaggaa
 atgaatcctc ctgataacat caaggataca aaaagtgaca tcatattctt
 tcagagaagt gtcccaggac atgataataa gatgcaattt gaatcttcat
 catacgaagg atactttcta gcttgtgaaa aagagagaga cctttttaa
 ctcatcttga aaaaagagga tgaattgggg gatagatcta taatgttcac
 tgttcaaaac gaagactag

tactttggca agcttgaatc taaattatca gtcataagaa atttgaatga ccaagttctc 60
 ttcattgacc aaggaaatcg gcctctatct gaagatatga ctgattctga ctgtagagat 120
 aatgcacccc ggaccatatt tattataagt atgtataaag atagccagcc tagaggatg 180
 gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc tctcctgtga gaacaaaatt 240
 atttccttta aggaaatgaa tcctcctgat aacatcaagg atacaaaaag tgacatcata 300
 ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcatacata 360
 gaaggatact ttctagcttg tgaaaaagag agagaccttt ttaaactcat tttgaaaaaa 420
 gaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga ctag 474

: (SEQ ID NO:69)

MAWTVLLGLLSHCTGSVTSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS
 MYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRSVPGHDNKMQFESSY
 EGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNE

Met Ala Trp Thr Val Leu Leu Leu Gly Leu Leu Ser His Cys Thr Gly
 1 5 10 15

Ser Val Thr Ser Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile
 20 25 30

Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro
 35 40 45

Leu Phe Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg
 50 55 60

Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met
 65 70 75 80

Ala Val Thr Ile Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys
 85 90 95

Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile
 100 105 110

Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly
 115 120 125

His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe
 130 135 140

Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys
 145 150 155 160

Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu
 165 170 175

Asp

: (SEQ ID NO:70)

MAWTVLLGLLSHCTGSVTS

Met Ala Trp Thr Val Leu Leu Leu Gly Leu Leu Ser His Cys Thr Gly
 1 5 10 15

Ser Val Thr Ser
 20

: (SEQ ID NO:71)

YFGKLESKLSVIRNLNDQVLFDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEK
 ISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRSVPGHDNKMQFESSYEGYFLACEKERDLFKLILKK
 EDELGDRSIMFTVQNE

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
 1 5 10 15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
 20 25 30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
 35 40 45

Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
 50 55 60

Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
 65 70 75

Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
 85 90 95

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
 100 105 110

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
 115 120 125

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
 130 135 140

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
 145 150

Several changes could be made in IL-18, e.g., as presented herein. Changes in non-surface exposed residues that could be made that would result in the high probability of retention of IL-18 activity with no changes in immunogenicity are:

Thr¹⁰ for Ser¹⁰
 Val¹² for Ile¹²
 Ser⁴⁵ for Thr⁴⁵
 Tyr⁴⁷ for Phe⁴⁷
 Phe⁵² for Tyr⁵²
 Val⁶⁴ for Ile⁶⁴
 Tyr¹⁰¹ for Phe¹⁰¹

These compounds would be useful as IL-18 agonists, for raising anti-IL-18 antibodies, for assays for IL-18 or IL-18 binding proteins and for preparation of affinity columns for the purification of IL-18 binding proteins.

Changes in amino acids with a low percentage of surface exposure that could be made that would result in the high probability of retention of IL-18 activity with possible changes in immunogenicity are:

Val⁵ for Leu⁵
 Val²⁰ for Leu²⁰
 Ile²⁰ for Leu²⁰
 Tyr²¹ for Phe²¹
 Val²² for Ile²²
 Ile⁶⁶ for Val⁶⁶
 Thr⁷² for Ser⁷²
 Phe¹⁴⁸ for Ser¹⁴⁸

These compounds would be useful as IL-18 agonists, for raising anti-IL-18 antibodies, for assays for IL-18 or IL-18 binding proteins and for preparation of affinity columns for the purification of IL-18 binding proteins.

Changes that could be made in amino acids involved in receptor contact that would result in alteration of IL-18 activity by either increasing or decreasing binding of the IL-18 analog to the IL-18 receptor are:

Glu⁴ for Lys⁴
 Ile⁶ for Glu⁶
 Asp⁸ for Lys⁸
 Ile¹³ for Arg¹³
 Arg¹⁵ for Leu¹⁵
 Lys¹⁷ for Asp¹⁷
 Lys²⁷ for Arg²⁷
 Ala³⁰ for Phe³⁰
 Lys³⁵ for Asp³⁵
 Phe³⁷ for Asp³⁷
 Glu³⁸ for Cys³⁸
 Ala³⁹ for Arg³⁹
 Trp⁴⁰ for Asp⁴⁰
 Glu⁵¹ for Met⁵¹
 Gly⁵³ for Lys⁵³
 Ile⁵⁶ for Gln⁵⁶
 Ala⁵⁸ for Arg⁵⁸
 Lys⁶² for Val⁶²
 Lys⁹⁴ for Asp⁹⁴
 Phe⁹⁵ for Thr⁹⁵
 Leu¹⁰⁴ for Arg¹⁰⁴
 Ile¹⁰⁸ for Gly¹⁰⁸

Lys¹¹¹ for Asn¹¹¹
 Phe¹²⁹ for Lys¹²⁹
 Asp¹³¹ for Arg¹³¹
 Leu¹³² for Asp¹³²
 Glu¹³³ for Leu¹³³
 Ala¹³⁴ for Phe¹³⁴
 Thr¹⁵⁰ for Met¹⁵⁰
 Ser¹⁵¹ for Phe¹⁵¹

Depending on the alteration of receptor binding or receptor activity, these compounds would be useful as IL-18 agonists or antagonists, for preparation of antibodies against IL-18, in assays for IL-18 or IL-18 binding proteins and the preparation of affinity columns for the purification of IL-18 binding proteins.

3. Other claimed changes in mature human IL-18 protein sequence:

- a. Human sequence reference AF380360-1, linked to either signal sequence listed above, with the following sequence of mature human IL-18; this appears to be a natural variant of human IL-18, with changes in blue.

: (SEQ ID NO:72)

YFGKLESK LSVIRNLNNQVLFDIQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISV
 KCEKISTLSCENKIISFKEVNPDPNIDTKSDIIFQRSVPGHDNKMQFESSSYEGYF
 LTCEKERDLFKLILKKEDELGDRSIMFTVQNE

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	1	5	10	15
Asn	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	20	25	30	
Met	Thr	Asp	Ser	Asp	Cys	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	35	40	45	
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	50	55	60	
Ser	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile	65	70	75	80
Ile	Ser	Phe	Lys	Glu	Val	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	85	90	95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	100	105	110	
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Thr	Cys	Glu	115	120	125	
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	130	135	140	
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				145	150	155	

(SEQ ID NO:73)

```

tactttggca agcttgaatc taaattatca gtcataagaa atttgaataa ccaagttctc 60
ttcattgacc aaggaaatcg gcctctatct gaagatatga ctgattctga ctgtagagat 120
aatgcacccc ggaccatatt tattataagt atgtataaag atagccagcc tagaggtatg 180
gctgtaacta tctctgtgaa gtgtgagaaa atttcaactc tctcctgtga gaacaaaatt 240
atttccttta aggaagtga tctcctgat aacatcaagg atacaaaaag tgacatcata 300
ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcatacatc 360
gaaggatact ttctaacttg tgaaaaagag agagaccttt ttaaactcat tttgaaaaaa 420
gaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga ctag 474

```

b. Human sequence reference AAC27787; this appears to be a natural variant of human IL-18. Only mature human IL-18 protein is shown, DNA sequence is not available from database:

```

yfgklesklsvirnlndqvlfidqgnrplledmtsdscrdnaprtifiirmkykdsqprgmavtisvkcek
istlscenkiisfkemppdnikdtkdsdiiffqrsvpghdnkmqfesssyegyflacekerdlfklikk
edelgdrsimftvqsed

```

(SEQ ID NO:74)

```

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1           5           10           15

```

```

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Leu Glu Asp
20           25           30

```

```

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35           40           45

```

```

Ile Arg Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50           55           60

```

```

Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65           70           75           80

```

```

Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85           90           95

```

```

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100          105          110

```

```

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
115          120          125

```

```

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
130          135          140

```

```

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Ser Glu Asp
145          150          155

```

c. Macaque sequence reference AF303732; mature macaque protein and DNA sequences are shown, and would be linked to either signal sequence shown above. Blue residues are altered from human consensus sequence:

(SEQ ID NO:75)

YFGKLESKLSIIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIINMYKDSQPRGMAVAISV
KCEKISTLSCENRIISFKEMNPPDNIKDTKSDIIFQRSVPGHDNKMQFESSSYEGYFLACEKERDLYKL
ILKKKDELGDRSIMFTVQNE

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Ile Ile Arg Asn Leu Asn
1 5 10 15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45

Ile Asn Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Ala Ile
50 55 60

Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Arg Ile
65 70 75 80

Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85 90 95

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100 105 110

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
115 120 125

Lys Glu Arg Asp Leu Tyr Lys Leu Ile Leu Lys Lys Lys Asp Glu Leu
130 135 140

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145 150 155

(SEQ ID NO:76)

tactttggca agcttgaatc taaattatca atcataagaa atttgaatga ccaagttctc 60
ttcattgacc aaggaaatcg gccctatatt gaagatatga ctgattctga ctgtagagat 120
aatgcacccc ggaccatatt tattataaat atgtataaa atagccagcc tagagggtatg 180
gctgtagcca tctctgtgaa atgtgagaaa atttcaactc tctcctgtga gaacagaatt 240
atttccttta aggaaatgaa tctcctgat aacatcaagg atacgaaaag tgacatcata 300
ttctttcaga gaagtgtccc aggacatgat aataagatgc aatttgaatc ttcacatac 360
gaaggatact ttctagcttg tgaaaaagag agagaccttt ataaactcat tttgaaaaaa 420
aaggatgaat tgggggatag atctataatg ttcactgttc aaaacgaaga ctg 474

Reference: *J Interferon Cytokine Research* 21:173-180, 2001, LD
Giavedoni et al

- d. Mutant human IL-18 with increased IL-18 activity and reduced ability to be inhibited by IL-18 binding protein; mature human IL-18 sequence with two altered residues indicated in blue:

(SEQ ID NO:77)

YFGKLASKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYADSQPRGMAVTISVKCEK
ISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKK
EDELGDRSIMFTVQNE

```

Tyr Phe Gly Lys Leu Ala Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1           5           10           15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20           25           30
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35           40           45
Ile Ser Met Tyr Ala Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50           55           60
Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65           70           75           80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85           90           95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100          105          110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
115          120          125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
130          135          140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145          150          155

```

Reference: PNAS 98:3304-3309, 2001 SM Kim et al.

Accordingly, based on the above non-limiting examples of specific substitutions, alternative substitutions can be made by routine experimentation, to provide alternative tumor/adjuvant vaccines of the present invention, e.g., by making one or more substitutions, insertions or deletions in proteins or tumor proteins which give rise to effective immune responses.

Amino acid sequence variations in a tumor protein or cytokine of the present invention can be prepared e.g., by mutations in the DNA. Such tumor or cytokine variants include, for example, deletions, insertions or substitutions of nucleotides coding for different amino acid residues within the amino acid sequence. Obviously, mutations that will be made in nucleic acid encoding a tumor protein or cytokine must not place the sequence out of reading frame and preferably will not create complementary domains that could produce secondary mRNA structures (see, e.g., Ausubel (1995

rev.), *infra*; Sambrook (1989), *infra*).

Tumor protein or cytokine-encoding nucleic acid of the present invention can also be prepared by amplification or site-directed mutagenesis of nucleotides in DNA or RNA encoding a tumor or cytokine protein or portion thereof, and thereafter synthesizing or reverse transcribing the encoding DNA to produce DNA or RNA encoding a tumor protein or cytokine variant (see, e.g., Ausubel (1995 rev.), *infra*; Sambrook (1989), *infra*), based on the teaching and guidance presented herein.

Recombinant viruses expressing tumor/adjuvant proteins of the present invention, or nucleic acid vectors encoding therefor, include a finite set of tumor/adjuvant-encoding sequences as substitution nucleotides that can be routinely obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein. For a detailed description of protein chemistry and structure, see Schulz, G. E. et al., *Principles of Protein Structure*, Springer-Verlag, New York, N.Y. (1978), and Creighton, T. E., *Proteins: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco, Calif. (1983), which are hereby incorporated by reference. For a presentation of nucleotide sequence substitutions, such as codon preferences, see Ausubel et al., eds, *Current Protocols in Molecular Biology*, Greene Publishing Assoc., New York, N.Y. (1987-2001) (hereinafter, "Ausubel et al, sections A.1.1-A.1.24, and Sambrook, J. et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) at Appendices C and D.

Thus, one of ordinary skill in the art, given the teachings and guidance presented herein, will know how to substitute other amino acid residues in other positions of an DNA or RNA to obtain alternative tumor/adjuvant vaccines, including substitutional, deletional or insertional variants.

EXAMPLES

Screening Assays for Tumor Activity

For screening anti-tumor activity of sera or cells from an individual immunized with a vaccine of the invention, any known and/or suitable screening assay can be used, as is known in the art.

Specific Embodiment: Recombinant Vaccinia Virus Encoding tumor/adjuvant's, Nucleic acid vaccines and Methods of Making and Using Thereof

Overview. A suitable recombinant viral vector is used according to the present invention for expressing tumor proteins (e.g., MUC-1, PSA, KLK3 or any portion, variant or combination thereof) to provide at least a portion of a vaccine useful for the production, testing or use of a tumor vaccine of the present invention that induces at least one of a humoral or cellular immune response against the tumor, a portion thereof or a cell thereof, as well as for analyses of B-cell and CTL determinants.

A tumor vaccine of the present invention expresses at least one tumor nucleic acid or protein (tumor/adjuvant) and at least one adjuvant nucleic acid or protein. The tumor vaccine functionally encodes at least one tumor/adjuvant or adjuvant. Multiple, distinct fragments or plasmids encoding tumor/adjuvant and/or adjuvant (e.g., IL-18) can be prepared by substituting one tumor/adjuvant encoding sequence with another, e.g., using a restriction fragment or mutagenesis, according to known methods (see, e.g., Ausubel or Sambrook, *supra*).

Preparation of Tumor Vaccine. Methods for the preparation of individual plasmids (each expressing at least one unique tumor or adjuvant protein sequence) can utilize DNA or RNA amplification for the substitution of isolated protein variant sequences into a vector, which vector encodes a known tumor and/or adjuvant protein sequence, as known in the art.

Methods of amplification of RNA or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein. Known methods of DNA or RNA amplification include, but are not limited to polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202,

4,800,159, 4,965,188, to Mullis et al.; U.S. Pat. Nos. 4,795,699 and 4,921,794 to Tabor et al; U.S. Pat. No. 5,142,033 to Innis; U.S. Pat. No. 5,122,464 to Wilson et al.; U.S. Pat. No. 5,091,310 to Innis; U.S. Pat. No. 5,066,584 to Gyllensten et al; U.S. Pat. No. 4,889,818 to Gelfand et al; U.S. Pat. No. 4,994,370 to Silver et al; U.S. Pat. No. 4,766,067 to Biswas; U.S. Pat. No. 4,656,134 to Ringold) and RNA mediated amplification which uses anti-sense RNA to the target sequence as a template for double stranded DNA synthesis (U.S. Pat. No. 5,130,238 to Malek et al, with the trade name NASBA), the entire contents of which patents are herein entirely incorporated by reference.

For example, recombinant tumor vaccine constructs prepared by this route can be used for immunizations and elicitation of tumor-specific T and/or B-cell responses. Primers utilize conserved tumor sequences and thus successfully amplify genes from many diverse tumor patient or cell samples or from tumor nucleic acid libraries, as non-limiting examples. The basic techniques described here can similarly be used with PCR or other types of amplification primers, in order to substitute smaller or larger pieces of the sequence from field isolates for that found in vectors encoding a tumor protein. See, e.g., Ausubel; *supra*, Sambrook, *supra*.

Tumor/Adjuvant Encoding Nucleic Acids. The technique can use, as a non-limiting example, the isolation of DNA from tumor infected cells and the amplification of sequences by PCR. PCR or other amplification products provide the simplest means for the isolation of tumor sequences, but any other suitable and known methods can be used such as cloning and isolation of tumor/adjuvant encoding nucleic acid or proteins (see Ausubel, *infra*; Sambrook, *infra*). Enzyme restriction sites are preferably incorporated into PCR or other amplification primer sequences to facilitate gene cloning.

Isolated DNA for PCR can be prepared from multiple tumor or adjuvant sources, inclusive of fresh or frozen whole blood or tumor tissue or cells from tumor+ patients and cells that have been infected in vitro with tumor virus isolates.

In order to produce new tumor/adjuvant constructs, the polymerase chain reaction (PCR) is preferably used to amplify 100-2700 base pairs (bp) of a tumor protein encoding nucleic acid from each different tumor patient, tissue or cell sample. The PCR primers can represent well-conserved tumor sequences which are suitable for amplifying genes from known samples of genes, isolated tumors or diverse tumor patient samples. The amplified DNA preferably comprises a portion encoding 10-900 (such as 100-400, 400-600 or 600-900, or any range or value therein) amino acids of a PSA, MUC-1 or KLK-3 protein. Preferably, most or all of the entire gene is amplified. Optionally, the MUC-1 encoding sequence amplified is missing part or all of sequences encoding the 20 amino acid repeat or any combination or number of copies thereof, such but not limited, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 copies or any fraction thereof, such .1, .2, .3, .4, .5, .6, .7, .8, .9 of the encoding nucleic acid repeat, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 amino acids or any combination thereof. Non-limiting examples include 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, and the like, including any fractional amount thereof, such as .1, .2, and the like.

The PCR primers can be designed so that restriction enzyme sites flank the tumor protein or cytokine adjuvant gene sequence in a suitable expression plasmid or vector, such that they are incorporated into the amplified DNA products. Suitable host cells can then be transformed with the tumor/adjuvant plasmid(s) via any of a number of methods well-known in the art, including, e.g., electroporation, and recombinant colonies are picked and examined by sequencing.

Methods for the production of expression vectors are well-known in the art (see, e.g., Mackett, M. et al., Proc. Natl. Acad. Sci. (USA) 79:7415-7419 (1982); Panicali, D., and Paoletti, E., Proc. Natl. Acad. Sci. (USA) 79:4927-4931 (1982); U.S. Pat. No. 4,169,763; Mazzara, G. P. et al., Methods in Enz. 217:557-581 (1993), Ausubel et al., *infra*, (e.g., 16.15-16.19), each of which are entirely incorporated herein by reference).

For use in the present invention a nucleic acid vaccine or a viral vector vaccine can be either used alone, in combination or sequentially.

As a non-limiting example of a suitable viral vector for a tumor vaccine of the present invention, vaccinia virus has a number of useful characteristics, including capacity that permits cloning large fragments of foreign DNA (greater than 20 Kb), retention of infectivity after insertion of foreign DNA, a wide host range, a relatively high level of protein synthesis, and suitable transport, secretion, processing and post-translational modifications as dictated by the primary structure of the expressed protein and the host cell type use. For example, N-O-glycosylation, phosphorylation, myristylation, and cleavage, as well as assembly of expressed proteins, occur in a faithful manner.

Several variations of the vaccinia vector have been developed and are suitable for use in the present invention (e.g., see Ausubel et al., *infra*, sec. 16.15-16.19). Most commonly, after obtaining the virus stock (Ausubel, *infra* at sec. 16.16), a nucleic acid sequence encoding a tumor/adjuvant is placed under control of a vaccinia virus promoter and integrated into the genome of vaccinia so as to retain infectivity (Ausubel et al., *infra* at sec. 16.17). Alternatively, expression can be achieved by transfecting a plasmid containing the vaccinia promoter-controlled gene encoding a tumor/adjuvant into a cell that has been infected with wild-type vaccinia.

Preferably, the host cell and vector are suitable and approved for use in vaccination of mammals and humans. These recombinant vectors are then characterized using various known methods (Ausubel et al., *infra* at sec. 16.18). In still another variation, the bacteria phage T7 RNA polymerase chain can be integrated into the genome of the vector so that the tumor/adjuvant encoding sequences will be expressed under the control of a T7 promoter, either in transfected plasma, plasmid or a recombinant vaccinia virus, will be expressed.

The use of pox virus promoters is preferred for vaccinia expression because cellular and other viral promoters are not usually recognized by the vaccinia transcriptional apparatus. A compound early/late promoter is preferably used in recombinant vaccinia for nucleic acid vaccines, as it is desirable to express the tumor/adjuvant as an antigen

that is presented in recombinant vaccinia virus infected host cell in association with major histocompatibility class (MHC) I or II. Such MHC associated tumor protein will then form cytotoxic T cell targets, and prime vaccinated mammals for a cytotoxic T cell response and/or a humoral response against the expressed tumor tumor/adjuvants. This is because the ability of vaccinia viral vectors to induce MHC presentation in host cells for this type of antigen appears to diminish late in the infection stage. Transcripts originating early will terminate after the sequence TTTTNT and lead to inadequate MHC presentation.

Alternatively, any such termination motifs within the coding sequence of the gene can be altered by mutagenesis if an early pox virus promoter is used, in order to enhance MHC presentation of protein antigens in host cells (Earl et al., *infra*, 1990). To mimic vaccinia virus mRNAs, untranslated leader and 3'-terminal sequences are usually kept short, if they are used in the vaccinia plasmids incorporating tumor/adjuvant encoding sequences.

Preferably, the plasmid used for making vaccinia constructs according to the present invention has been designed with restriction endonuclease sites for insertion of the gene downstream of the vaccinia promoter (Ausubel et al., *infra*, sec. 16.17). More preferably, the plasmid already contains an protein encoding sequence, wherein the restriction sites occur uniquely near each of the beginning and ends of the protein coding sequence. The same restriction fragment of the tumor/adjuvant encoding sequence can then replace the corresponding sequence in the plasmid. In such cases, the major portion of the tumor/adjuvant encoding sequence can be inserted after removing most or all of the protein encoding sequence from the plasmid.

Preferably, the resulting vaccinia construct (containing the tumor/adjuvant encoding sequence and the vaccinia promoter) is flanked by vaccinia DNA to permit homologous recombination when the plasmid is transfected into cells that have been previously infected with wild-type vaccinia virus. The flanking vaccinia virus DNA is chosen so that the recombination will not interrupt an essential viral gene.

Without selection, the ratio of recombinant to parental vaccinia virus is usually about

1:1000. Although this frequency is high enough to permit the use of plaque hybridization (see Ausubel et al., *infra* at sec. 6.3 and 6.4) or immunoscreening (Ausubel et al., *infra* at sec. 6.7) to pick recombinant viruses, a variety of methods to facilitate recombinant-virus identification have been employed. Nonlimiting examples of such selection or screening techniques are known in the art (see Ausubel et al., *infra* at sec. 16.17). Usually, the expression cassette is flanked by segments of the vaccinia thymidine kinase (TK) genes so that recombination results in inactivation of TK. Virus with a TK.sup.- phenotype can then be distinguished from those with a TK.sup.+ phenotype by infecting a TK.sup.- cell line in the presence of 5-bromo-deoxyuridine (5-BrdU), which must be phosphorylated by TK to be lethally incorporated into the virus genome. Alternatively or additionally, recombinant viruses can be selected by the co-expression of a bacterial antibiotic resistant gene such as ampicillin (amp) or guanine phosphoribosyl transferase (gpt). As a further example, co-expression of the *Escherichia coli* lac Z gene allows co-screening of recombinant virus plaques with Xgal (Ausubel, *infra*, sec. 16.17).

The recombinant vaccinia viruses expressing a tumor/adjuvant of the present invention can be optionally attenuated or inactivated according to known methods, such as by heat, paraformaldehyde treatment, ultraviolet irradiation, propiolactone treatment, hybrid or chimera formation or by other known methods (see, e.g., Zagury et al., *Nature* 332:728-731 (1988); Ito et al., *Cancer Res.* 50:6915-6918 (1990); Wellis et al., *J. Immunol.* 99:1134-9 (1967); D'Honcht, *Vaccine* 10 (Suppl.):548-52 (1992); Selenka et al., *Arch. Hyg. Bakteriol.* 153:244-253 (1969); Grundwald-Bearch et al., *J. Cancer Res. Clin. Oncol.* 117:561-567 (1991); the contents of which are entirely incorporated here by reference). For example, heat inactivation at 60.degree. C. will reduce virus titer considerably. Such attenuation techniques are safety tested, as incomplete inactivation might result in patient death (Dorozynski and Anderson, *Science* 252:501-502 (1991)).

Such attenuated or inactivated recombinant vaccinia is to be used where the patient may have a compromised immune system as complications or death can occur when live vaccinia is administered.

Pharmaceutical Compositions

Pharmaceutical preparations of the present invention, suitable for inoculation or for parenteral or oral administration, include a polyrecombinant virus vaccine comprising of at least 4, and up to about 10,000, preferably 4 to about 1000, and more preferably about 10 to about 100 different recombinant viruses, in the form of a cell lysate, membrane-bound fraction, partially purified, or purified form. Preferably, the nucleic acid vaccine comprises recombinant virus containing cell lysate (or membrane-bound fractions thereof) that further comprise tumor/adjuvant proteins already expressed by the recombinant viruses. The inclusion of the expressed tumor/adjuvants is now discovered to enhance the primary antibody response.

The nucleic acid vaccine composition can be in the form of sterile aqueous or non-aqueous solutions, suspensions, or emulsions, and can also contain auxiliary agents or excipients which are known in the art. Each of the at least about 4-20 different viruses encode and express a different tumor/adjuvant, as presented herein. tumor/adjuvants encoding DNA can be selected to represent tumor/adjuvants suitable for treatment. For example, a vaccine could represent sequences from any or any combination of suitable tumors and adjuvant proteins.

A nucleic acid vaccine composition can further comprise immunomodulators such as cytokines which accentuate an immune response to a viral infection. See, e.g., Berkow et al., eds., *The Merck Manual*, Fifteenth Edition, Merck and Co., Rahway, N.J. (1987); Goodman et al., eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, Eighth Edition, Pergamon Press, Inc., Elmsford, N.Y. (1990); Avery's *Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, Third Edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, Md. (1987); and Katzung, ed. *Basic and Clinical Pharmacology*, Fifth Edition, Appleton and Lange, Norwalk, Conn. (1992), which references and references cited therein, are entirely incorporated herein by reference as they show the state of the art.

As would be understood by one of ordinary skill in the art, when a nucleic acid vaccine of the present invention is provided to an individual, it can be in a composition which

can further comprise at least one of salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the composition. Adjuvants are substances that can be used to specifically augment at least one immune response. Normally, the adjuvant and the composition are mixed prior to presentation to the immune system, or presented separately, but into the same site of the being immunized. Adjuvants can be loosely divided into several groups based upon their composition. These groups include oil adjuvants, mineral salts (for example, $\text{AlK}(\text{SO.sub.4}).\text{sub.2}$, $\text{AlNa}(\text{SO.sub.4}).\text{sub.2}$, $\text{AlNH.sub.4}(\text{SO.sub.4})$, silica, kaolin, and carbon), polynucleotides (for example, poly IC and poly AU nucleic acids), and certain natural substances (for example, wax D from *Mycobacterium tuberculosis*, substances found in *Corynebacterium parvum*, or *Bordetella pertussis*, and members of the genus *Brucella*). Among those substances particularly useful as adjuvants are the saponins (e.g., Quil A., Superfos A/S, Denmark). Examples of materials suitable for use in vaccine compositions are disclosed, e.g., in Osol, A., ed., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa. (1980), pp. 1324-1341, which reference is entirely incorporated herein by reference.

A pharmaceutical vaccine composition of the present invention can further or additionally comprise at least one antiviral chemotherapeutic compound. Non-limiting examples can be selected from at least one of the group consisting of gamma globulin, amantadine, guanidine, hydroxy benzimidazole, interferon-.alpha., interferon-.beta., interferon-.gamma., interleukin-16 (IL-16; Kurth, *Nature*, Dec. 8, 1995); thiosemicarbazones, methisazone, rifampin, ribvirin, a pyrimidine analog (e.g., AZT and/or 3TC), a purine analog, foscarnet, phosphonoacetic acid, acyclovir, dideoxynucleosides, a protease inhibitor (e.g., saquinavir (Hoffmann-La Roche); indinavir (Merck); ritonavir (Abbott Labs); AG 1343 (Agouron Pharmaceuticals); VX-2/78 (Glaxo Wellcome)); chemokines, such as RANTES, MIP1.alpha. or MIP1.beta. (*Science* 270:1560-1561 (1995)) or ganciclovir. See, e.g., Richman: *AIDs Res. Hum. Retroviruses* 8: 1065-1071 (1992); *Annu Rev Pharmacol Toxicol* 33: 149-164 (1993); *Antimicrob Agents Chemother* 37: 1207-1213 (1993); *AIDs Res. Hum. Retroviruses* 10: 901 (1994); Katzung (1992), *infra*, and the references cited therein on pages 798-800 and 680-681, respectively, which references are herein entirely incorporated by reference.

Pharmaceutical Uses

The administration of a vaccine (or the antisera which it elicits) can be for either a "prophylactic" or "therapeutic" purpose, and preferably for prophylactic purposes. When provided prophylactically, the nucleic acid vaccine composition is provided in advance of any detection or symptom of tumor associated pathology. The prophylactic administration of the compound(s) serves to prevent or attenuate any subsequent tumor associated pathology.

When provided therapeutically, the nucleic acid or viral vaccine is provided upon the detection of a symptom of actual infection. The administration of a vaccine after detection of tumor-associated pathology is provided only where the patient's immune system is determined to be capable of responding to administration of a vaccine of the present invention.

Alternatively, where the patient's immune response is compromised, therapeutic administration preferentially involves the use of an attenuated or inactivated viral vaccine composition where the viral vaccines are attenuated or inactivated, as presented above. See, e.g., Berkow (1987), *infra*, Goodman (1990), *infra*, Avery (1987), *infra* and Katzung (1992), *infra*, Dorozynski and Anderson, Science 252:501-502 (1991) which are entirely incorporated herein by reference, including all references cited therein.

A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically or prophylactically effective amount" if the amount administered is physiologically significant. A vaccine or composition of the present invention is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient, preferably by enhancing a humoral or cellular immune response to a tumor.

The "protection" provided need not be absolute, i.e., the tumor need not be totally prevented or eradicated, provided that there is a statistically significant improvement

relative to a control population. Protection can be limited to mitigating the severity or rapidity of onset of symptoms of the disease.

Pharmaceutical Administration

A vaccine of the present invention can confer resistance to one or more types of a tumor. The present invention thus concerns and provides a means for preventing or attenuating infection by at least one tumor. As used herein, a vaccine is said to prevent or attenuate a disease if its administration to an individual results either in the total or partial attenuation (i.e. suppression) of a symptom or condition of the disease, or in the total or partial immunity of the individual to the disease.

At least one nucleic acid vaccine of the present invention can be administered by any means that achieve the intended purpose, using a pharmaceutical composition as described herein.

For example, administration of such a composition can be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal, or buccal routes. Subcutaneous administration is preferred. Parenteral administration can be by bolus injection or by gradual perfusion over time. See, e.g., Berkow (1987), *infra*, Goodman (1990), *infra*, Avery (1987), *infra*, and Katzung (1992), *infra*, which are entirely incorporated herein by reference, including all references cited therein.

A typical regimen for preventing, suppressing, or treating a disease or condition which can be alleviated by a cellular immune response by active specific cellular immunotherapy, comprises administration of an effective amount of a vaccine composition as described is above, administered as a single treatment, or repeated as enhancing or booster dosages, over a period up to and including one week to about 24 months.

According to the present invention, an "effective amount" of a vaccine composition is one which is sufficient to achieve a desired biological effect, in this case at least one of

cellular or humoral immune response to at least one tumor. It is understood that the effective dosage will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The ranges of effective doses provided below are not intended to limit the invention and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation. See, e.g., Berkow (1987), *infra*, Goodman (1990), *infra*, Avery (1987), *infra*, Ebadi, Pharmacology, Little, Brown and Co., Boston, Mass. (1985), and Katsung (1992), *infra*, which references and references cited therein, are entirely incorporated herein by reference. Whatever dosage is used, it should be a safe and effective amount as determined by known methods, as also described herein.

Subjects

The recipients of the vaccines of the present invention can be any mammal which can acquire specific immunity via a cellular or humoral immune response to tumor, where the cellular response is mediated by an MHC class I or class II protein. Among mammals, the preferred recipients are mammals of the Orders Primata (including humans, chimpanzees, apes and monkeys). The most preferred recipients are humans.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration, and are not intended to be limiting of the present invention.

Examples

We believe it is preferable that cytotoxic immunity to MUC1 be generated through the expression of MUC1 by antigen presenting cells with the subsequent presentation of digested MUC1 peptides in the context of Class I molecules. Transgene has taken an approach along these lines, using a vaccinia virus encoding MUC1 and IL-2 (29-31). This strategy would allow expression of MUC1 with natural processing of peptide for presentation to the immune system, with the function of IL-2 being to support

the growth of CTLs. In three of nine patients, cellular responses were detected, and the two patients with documented CTL activity survived the longest, although the results are not significant (31). One important limitation to this strategy is that repeated administration of a viral vector results in a strong immune response to the vector itself. This limits the number of times the drug can be administered, because the host immune response acts to clear the drug very quickly. Another approach that may make its way to the clinic, and appears effective in mice, is the fusion of MUC1+ tumor cells with dendritic cells, followed by vaccination of the mice with the fusion cells (32, 33). This leads to specific MUC1 cellular immunity that is protective for tumor challenge and tumor treatment in mice. Because every patient is immunologically unique, this would require unique reagents for each patient. This approach may thus turn out to be very difficult to translate into mass usage because of its expense and requirement for sophisticated medical expertise.

Our strategy is to use DNA vaccination to drive a cellular immune response against tumor cells expressing MUC1. We believe that this approach offers significant advantages over the other strategies listed above. First, DNA vaccines are known to generate strong humoral and cellular immune responses in numerous animal studies (34, 35), and cellular responses in at least one human trial (36). Second, we believe that a cellular immune response, with the generation of CTLs will be the best way to eliminate MUC1+ tumor cells. CTLs directed against a particular antigen recognize specific peptides presented in the context of Class I molecules on a cell surface. Recognition by CTL then results in destruction of the cell expressing that antigen. DNA vaccines can induce the generation of CTLs directed against the antigen encoded by the vaccine (34, 35). If the antigen is a tumor antigen, tumor cells would be lysed by the CTLs. In contrast, anti-tumor antibodies are typically of low avidity and are not very effective in causing ADCC of tumor cells. Third, by injecting a plasmid that will encode the whole MUC1 protein, the patient's immune system can choose the best peptides for presentation according to his/her unique array of Class I molecules, rather than limiting the drug to one or several putative Class I peptides. Fourth, we have shown in preclinical studies that a combination of plasmids encoding MUC1 and the cytokine IL-18 protect mice from developing tumors, whereas plasmids encoding MUC1 or IL-18 alone offer little to no protection. IL-18 is a cytokine known to skew a nascent immune response toward a cellular response, rather than a humoral response (37). Fifth, DNA vaccination is a

flexible therapeutic strategy, in that one can design a DNA vaccine that encodes not just MUC1 but other molecules that could help to drive the immune response. Sixth, DNA vaccines are simple in concept and delivery to the patient, and should provide a cost-effective approach toward cancer treatment. Seventh, DNA vaccines can be administered indefinitely to the patient, because DNA is nontoxic, and because only the protein product of the DNA, not the DNA itself, is immunogenic.

The invention is a plasmid that encodes human MUC1 and a plasmid that encodes human IL-18, or a multicistron plasmid that encodes both genes. The mode of delivery could also be MUC1 DNA and IL-18 DNA encoded by a viral vector, or RNA encoding each gene. The invention includes an IL-18 gene construct comprised of mature IL-18 linked to a heterologous signal sequence, specifically an immunoglobulin signal sequence. This permits mature IL-18 to be expressed without the requirement for caspase cleavage of the IL-18 precursor protein.

Coinjection of both MUC1 and IL-18 plasmids intramuscularly at the same site is presumed to cause the local expression of both proteins in muscle cells, as well as the takeup and expression of both plasmids by professional antigen presenting cells (APCs) that are migrating through the tissue. This leads to a memory immune response that is protective for animals subsequently challenged with MUC1⁺ tumor cells. It appears that the vaccination can break self-tolerance to MUC1.

The vaccination also leads to protection from subsequent challenge by MUC1⁻ tumor cells that are otherwise identical to the MUC1⁺ tumor cells. This phenomenon is known as epitope spreading, and may be a critical, unique feature of the vaccine that enables the immune system to develop a response to MUC1 and to other undefined antigens expressed by the tumor. Tumors are adept at evading the immune system, notably by changing their array of antigens on the cell surface (escape variants). Thus, a vaccine that induces immunity to more than one tumor antigen should make it more difficult for tumors to evade the immune system, and this could result in more effective cancer therapy.

Our studies show that MUC1 and IL-18 plasmids synergize to induce the formation of a protective anti-tumor immune response. The first study was performed in C57Bl/6 mice (43). Nine groups of animals were vaccinated with either vehicle control, empty vector, pMUC1, or pIL-18, singly or in combination. Three vaccinations were performed over a three-week period, and the mice were challenged with syngeneic

MUC1⁺ tumor cells (38, 39) by subcutaneous injection in the fourth week. Animals were then monitored for tumor incidence and tumor volume for up to seven weeks thereafter. Results are shown in Figure 1. None of the mice in the groups receiving vehicle, empty plasmid or pIL-18 were protected from developing tumors. Two groups received suboptimal doses of pMUC1, and only 2-3 mice were protected. Of the groups vaccinated with the various combinations of pMUC1 and pIL-18 plasmids, those groups receiving the higher dose of pMUC1 in combination with either dose of pIL-18 showed good protection (6/9 or 7/9 mice). These results are significantly different from the control results ($p=0.011$ or $p=0.003$).

Tumor volume was also evaluated. The best result was seen in the group receiving 5ug pMUC1/5ug pIL-18, where tumor growth appeared to be delayed to day 35. At that time the slope of tumor growth parallels that of the other groups (Figure 2).

Sera from the animals was collected pre-study, and at days 13, 26 and 34 during and after vaccination. Sera were tested for the presence of anti-MUC1 antibodies, but only low titers were seen. This result indicates that a strong anti-MUC1 antibody response was not responsible for the protection seen in the animals.

The surviving mice from the first phase of this study were then entered into a second phase, which was designed to learn if the mice had developed a protective anti-tumor immune response that could be recalled. The mice were subjected to a second challenge with MUC1⁺ tumor cells, with the results shown in Figure 3. Again, the group that originally received 5ug of each test plasmid fared well, with 4 of the original 9 mice protected for another 49 days, while in the group receiving 5ug pMUC1 and 50ug pIL-18, 3 of the original 9 mice were still protected. This result indicates that some of the rechallenged mice had developed a protective cellular immune response, because they were able to fend off a second challenge of tumor cells.

The above study showed that while neither plasmid alone offered much protection from tumor challenge, and thus did not prime the immune response particularly well, vaccination with both plasmids at certain doses could indeed lead to protection from tumor challenge, or at least a delay in tumor development. We then sought to reproduce these results in a model system more reflective of the human patient, and we used a strain of C57Bl/6 mice transgenic for human MUC1 (40-42; referred to as MUC1 Tg mice). This model would allow us to test if the combination of plasmids was effective, and if we could break tolerance to a self-antigen. We repeated the study shown above using the

transgenic mice and using increased doses of pMUC1, but testing the same doses of pIL-18.

The results in the second study are consistent with the first (44; see Figure 4). Animals receiving empty plasmid showed no protection from tumor challenge. Only one animal receiving the higher dose of pMUC1 was protected, while none of those receiving pIL-18 alone were protected. In contrast, the groups receiving the combinations of pMUC1/pIL-18 showed notable protection, particularly the group receiving the highest dose of each plasmid (8/9 without tumors; $p=0.002$).

On day 28 the tumors were excised and weighed, as shown in Figure 5. Neither the pMUC1 nor pIL-18 groups had mean weights that were significantly different from the empty vector control group. However, all four pMUC1/pIL-18 combination groups had mean tumor weights that were significantly smaller than those of the empty vector control group ($p=0.004-0.038$). The results show that not only did the combination of pMUC1/pIL-18 have a positive effect on tumor incidence, it had a positive effect on tumor weights as well. Neither of these effects was observed with either plasmid alone.

Mice from the combination groups were then rechallenged with MUC1⁺ tumor cells to learn if they had developed protective immunity that could be recalled (Figure 6). Of the 5 mice that had originally been vaccinated with 100ug pMUC1/50ug pIL-18, 4/5 remained free of tumor growths in phase II after the second tumor challenge. Both of the mice from the group that was vaccinated with 100ug pMUC1/5ug pIL-18 also remained free of growths throughout the second challenge, while 1 of 2 mice each from the two remaining groups developed growths. The results support the hypothesis that the mice developed a memory response that was recalled in response to the second tumor challenge.

We then determined if the mice had developed a broader immune response to antigens besides MUC1. The same animals in phase II were challenged again but with MUC1⁻ MC38 tumor cells. The MC38 cells are the parent line to the MUC1⁺ tumor cells, and are otherwise expected to be identical (38). Results of the third challenge are shown in Figure 7. Interestingly, the mice that were originally vaccinated with the 100ug dose of pMUC1 in combination with either dose of pIL-18 continue to be protected, while the three naïve control MUC1 Tg mice succumbed to tumors. This result suggests that the vaccinated mice have developed immunity to determinants shared between the two cell lines, in addition to immunity to MUC1. This phenomenon is known as epitope

spreading, and is well documented in autoimmune disease models in animals (46, 47). In these models, animals are first immunized with a self-protein or peptide against which they develop immunity, and the immune response causes the destruction of normal tissue expressing the native protein. After tissue destruction, the immune response broadens to include antigens that the animals were not immunized against but which are expressed by the target tissue. If such a process could be duplicated in humans, DNA vaccination could be very effective at inducing immunity to MUC1 as well as other unique determinants present on tumor cells, and broadening the immune response should only be helpful to patient therapy. In addition, tumor cells are continuously changing in response to environmental pressures, and therapy against one antigen could lead to remission until escape variants arise that no longer express that antigen. With epitope spreading, the immune response broadens to include other antigens and theoretically should improve the chances that the tumor cells will be unable to escape the vigilance of the immune system.

A second advantage of this approach includes the use of a human IL-18 construct that encodes the mature form of IL-18 linked to an immunoglobulin signal sequence. IL-18 is ordinarily expressed as a precursor protein that is not functional until it is cleaved into its mature form by caspase (48, 49). Most cells do not express caspase, therefore one strategy to ensure IL-18 expression in any cell type is to engineer the protein so that it does not require caspase cleavage for maturation. We have used a genomic fragment that encodes the anti-IL-12 12B75 heavy chain signal sequence (50) linked to a human IL-18 cDNA sequence to ensure production of human IL-18 in any cell type. This strategy was effective for both the human and mouse IL-18 genes.

A third advantage of our approach is to use a MUC1 cDNA that includes one of its own introns to improve expression from the plasmid (Figure 9).

A fourth advantage of our approach is the ability to encode more than one gene on a plasmid to enable delivery of more than one protein product to a target tissue/cell (51, 52). This should ensure that a target tissue expresses all desired proteins with the expectation of a more efficient induction of immune response. A double cistron vector has been constructed, and we have shown that it is capable of expressing mouse or human IL-12. IL-12 is a protein comprised of two subunits that must be co-expressed in the same cell in order for the mature molecule to be produced. The two protein subunits are encoded by different genes, and we have shown in tissue culture that a double cistron

vector encoding both genes results in more effective production of the mature protein than using two plasmids which encode either gene alone (51, 52).

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We wished to explore the epitope spreading phenomenon further, specifically to learn if DNA vaccination followed by just a single tumor challenge with MUC1+ cells

would give rise to epitope spreading. Animals were vaccinated according to the groups shown in Figure 10. Vaccination with pMUC1/pIL-18 is the only regimen that results in significant protection (8/18 mice) compared to the empty vector group ($p=0.007$). Tumor weights are likewise significantly smaller in this group versus the other three groups (Figure 11). These results confirm the previous data demonstrating that the combination of pMUC1 and pIL-18 offer better protection against tumor challenge, and also cause a significant reduction in tumor weight in those animals that still develop tumors. Further, the data indicate that the combination of the two plasmids allows one to break tolerance to the MUC1 self antigen in the MUC1 transgenic mice.

The 8 protected mice from the pMUC1/pIL-18 group, and the 3 protected mice from the pMUC1-only group were challenged with MUC1⁺ tumor cells (Figure 12). Only 1/15 control naïve animals survived tumor challenge, whereas 4/8 and 2/3 vaccinated animals remained tumor free. This result indicates that epitope spreading occurs with the immune response generated by the DNA vaccination and the first tumor challenge. Further, the fact that epitope spreading occurs in the pMUC1-only group suggests that IL-18 may not be required for this phenomenon to occur.

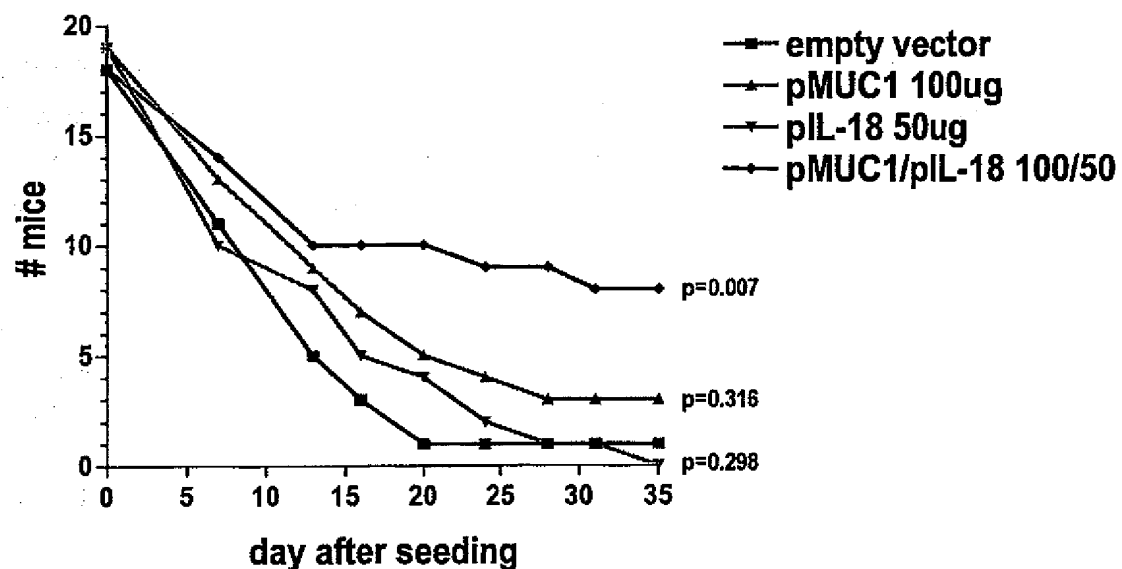


Figure 10. Tumor incidence in female MUC1 transgenic mice vaccinated with DNA as indicated in the legend, and subsequently challenged with MUC1⁺ tumor cells. Only

the group vaccinated with pMUC1/pIL-18 shows significantly improved protection from tumor challenge ($p=0.007$).

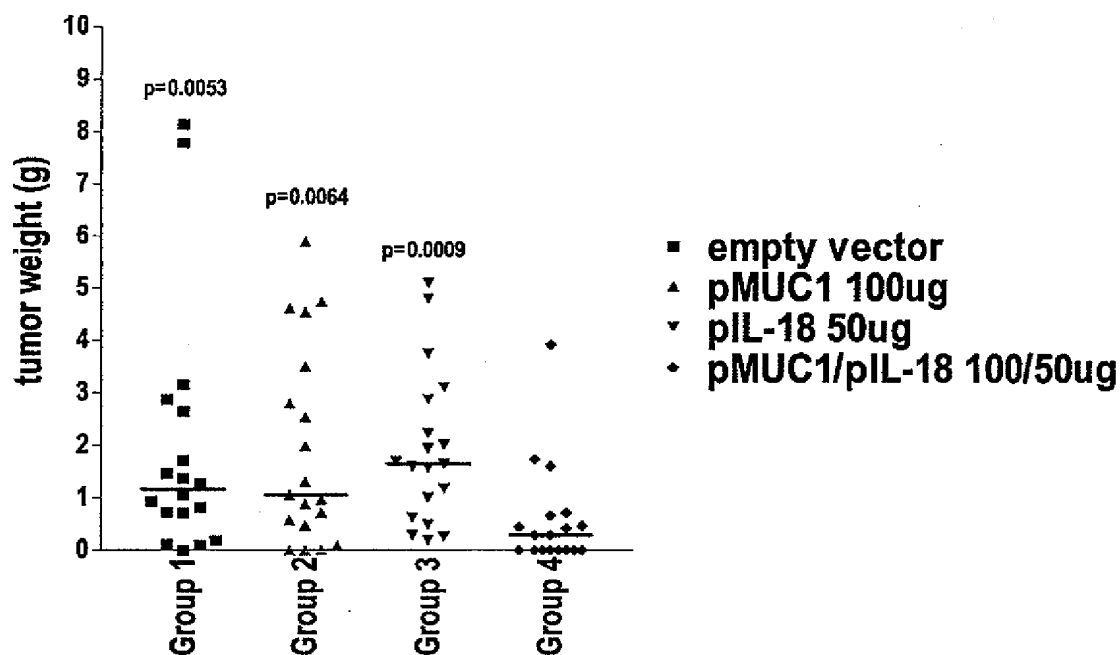


Figure 11. Media tumor weights at study end, from animals shown in Figure 1. Media tumor weight for group 4 is significantly different from those in the other groups.

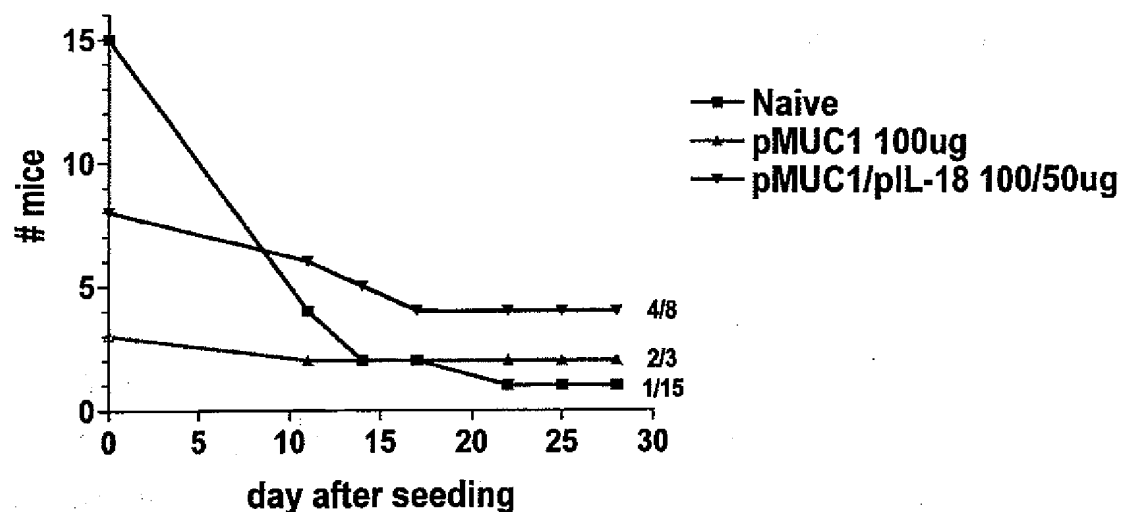


Figure 12. Rechallenge of protected mice from Figure 1 with MUC1⁺ tumor cells.

Experimental conditions for above: Female MUC1 transgenic mice were vaccinated in Figure 12 with the indicated quantities of plasmids, on day 0, 14, and 21. Mice were challenged with 1.5×10^5 MISA cells on day 28. They were monitored for tumor incidence, and tumor weights were measured at study end (Figure 11). The surviving mice from Figure 11 were challenged with 3×10^5 MC38 cells 45-47 days after the initial tumor challenge (Figure 12).

Tumor protection studies in male MUC1 transgenic mice

We have tested whether vaccination of male MUC1 transgenic mice with pMUC1 plasmid can induce a protective immune response upon challenge with MISA cells. Male mice were vaccinated on day 0, 14 and 21 with various doses of DNA, then challenged on Day 28 with 1.5×10^5 MISA tumor cells (Figure 13). In the control group, nearly all mice (9/10) succumbed to tumors. Male mice vaccinated with 150ug of pMUC1 showed good protection (6/10; $p=0.019$), and mice vaccinated with 100ug pMUC1 showed protection in 3/9 mice (not significant). Lower doses of pMUC1 did not result in any tumor protection. It appears that the pMUC1 plasmid alone can offer significant benefit in reducing tumor incidence, at high dose.

Tumor weights are shown in Figure 14. Again, the tumor weights in the highest dose group show a significant difference from the control group ($p=0.015$). This result suggests that the vaccination also helps to control growth of the tumor cells in the mice that still develop tumors.

To learn if the anti-tumor response was long-lived, the male mice that did not develop tumors (Figure 13) were rechallenged with 1.5×10^5 MISA cells on day 39 after the first tumor challenge. As shown in Figure 15, 3/6 and 1/3 of the pMUC1 vaccinated mice remained protected after the rechallenge, suggesting that some animals did develop a long-lived recall response to the tumors.

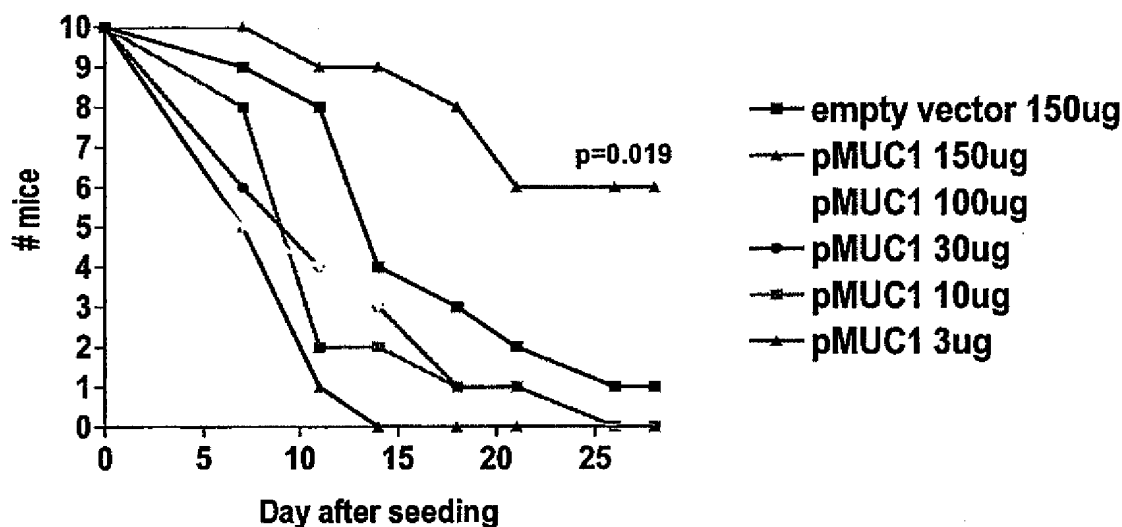


Figure 13. Tumor incidence in male mice vaccinated with pMUC1 or empty vector, followed by tumor challenge.

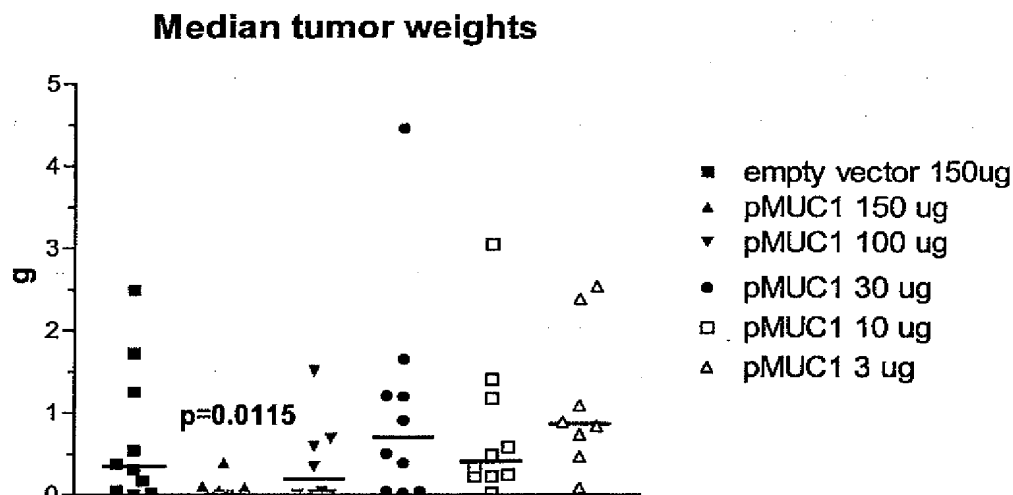


Figure 14. Tumor weights in male mice vaccinated with pMUC1.

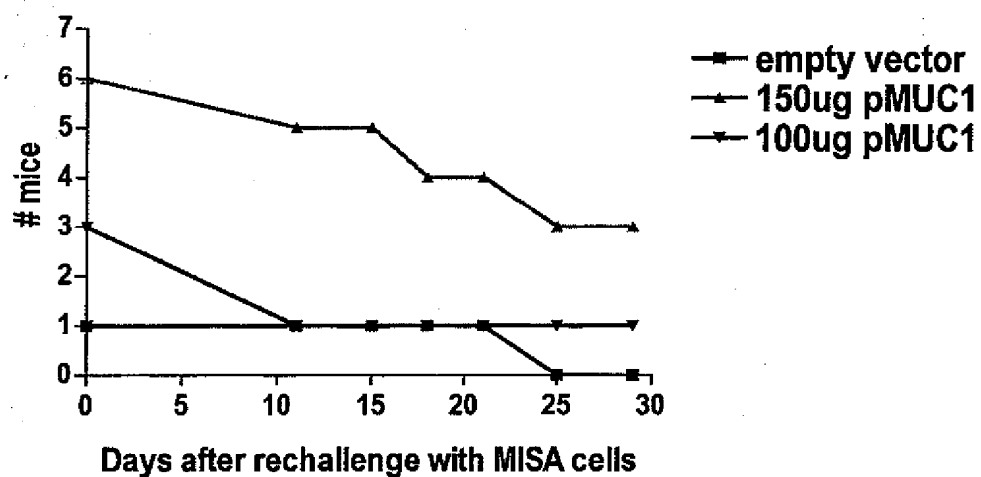


Figure 15. Tumor incidence in male mice rechallenged on the opposite flank with MUC1+ tumor cells.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

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WHAT IS CLAIMED IS:

1. A nucleic acid vaccine, comprising
 - (a) at least one polynucleotide encoding at least one antigenic portion of at least one amino acid sequence comprising or encoded by at least one of SEQ ID NOS:1-47 or variants thereof, or a nucleic sequence complementary thereto; and
 - (b) at least one polynucleotide encoding at least one adjuvant encoding portion of at least one amino acid sequence comprising or encoded by at least one of SEQ ID NOS:60-77 or variants thereof, or a sequence complementary thereto.
2. A nucleic acid vaccine according to claim 1, wherein said antigen is selected from at least one of MUC-1, PSA, or KLK2.
3. A nucleic acid vaccine according to claim 2, wherein said MUC-1 amino acid sequence is selected from at least one antigenic portion of at least one of SEQ ID NOS:20, 22, 26, 28, 30, 32, 34, 35, 37, 39, 41, 43, and 47.
4. A nucleic acid vaccine according to claim 2, wherein said PSA amino acid sequence is selected from at least one antigenic portion of at least one of SEQ ID NOS:1, 4-10, 12 and 14-15.
5. A nucleic acid vaccine according to claim 2, wherein said IL-18 amino acid sequence is selected from at least one antigenic portion of at least one of SEQ ID NOS:64, 65, 69, 70-71, 74-75 and 77.
6. A nucleic acid vaccine according to claim 1, wherein the vaccine further comprises at least one promoter sequence controlling the expression of said antigen encoding polynucleotide.
7. A nucleic acid vaccine according to claim 2, wherein the promoter is at least one cytomegalovirus immediate early (CMV) promoter.

8. A nucleic acid vaccine according to claim 2, wherein the promoter is at least one dihydrofolate reductase (dhfr) promoter.
9. A nucleic acid vaccine according to claim 2, where the promoter is at least one early or late SV40 promoter.
10. A nucleic acid vaccine according to claim 1, comprised of a nucleic acid vector.
11. A nucleic acid vaccine according to claim 1, comprised of a host cell
12. A nucleic acid vaccine according to claim 1, comprised of viral vector.
13. A composition comprising a nucleic acid vaccine according to claim 1.
14. A tumor/adjuvant vaccine composition comprising a nucleic acid vaccine according to claim 1 and a pharmaceutically acceptable carrier or diluent.
15. A nucleic acid vaccine composition of claim 11, further comprising an additional adjuvant and/or cytokine encoding sequence or component of the composition which enhances a nucleic acid vaccine immune response to at least one cancer associated tumor protein in a mammal administered the vaccine composition.
16. A method for eliciting an immune response to a cancer associated tumor protein in a mammal that is prophylactic for a cancer associated tumor protein, comprising administering to a mammal a nucleic acid vaccine according to claim 1.
17. A method for eliciting an immune response to a cancer associated tumor protein in a mammal for therapy of a tumor-associated pathology, comprising administering to a mammal a nucleic acid vaccine according to claim 1.
18. A method according to claim 13, further comprising priming or boosting a humoral or cellular immune response, or both, by administering an effective amount of at least one of said nucleic acid vaccine.
19. A method according to claim 14, further comprising priming or boosting a humoral or cellular immune response, or both, by administering an effective amount of at least one of said nucleic acid vaccine.

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(71) Applicant: **CENTOCOR, INC.** [US/US]; 200 Great Valley Parkway, Malvern, PA 19355 (US).

(72) Inventors: **SNYDER, Linda**; 1795 Honeysuckle Lane, Pottstown, PA 19465 (US). **SCALLON, Bernard**; 139 Hemlock Drive, Collegeville, PA 19426 (US). **KNIGHT, David, M.**; 2430 Whitehorse Road, Berwyn, PA 19312 (US). **MCCARTHY, Stephen, G.**; 701 Patrick Henry Circle, West Chester, PA 19382 (US). **GOLETZ, Theresa, J.**; 147 Pinecrest Drive, King of Prussia, PA 19406 (US). **BRANIGAN, Patrick, J.**; 223 Hansell Road, Lansdowne, PA 19050 (US).

(74) Agents: **JOHNSON, Philip, S.** et al.; One Johnson & Johnson Plaza, New Brunswick, NJ 08933 (US).

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- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **NUCLEIC ACID VACCINES USING TUMOR ANTIGEN ENCODING NUCLEIC ACIDS WITH CYTOKINE ADJUVANT ENCODING NUCLEIC ACID**

(57) Abstract: Nucleic acid vaccines are provided that comprise at least one tumor antigen encoding nucleic acid and at least one cytokine adjuvant encoding nucleic acid for prophylaxis or treatment of tumors. The viral vaccines of the invention are optionally combined or additionally administered with a recombinant virus or DNA vaccine booster.



WO 2003/031569 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/29640

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/70, 48/00; C12N 15/63; C07H 21/04

US CL : 514/44; 424/93.21; 435/320.1, 455; 536/23.1, 23.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 424/93.21; 435/320.1, 455; 536/23.1, 23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/61068 A1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 02 December 1999, see the entire document, particularly pages 3-5; page 12, lines 24-31; page 13, lines 17-25; and SEQ ID NO:1.	1-2, 4, 6-10, 12-14 and 16-19
X,P	WO 02/40059 A2 (AMERICAN FOUNDATION FOR BIOLOGICAL RESEARCH, INC.) 23 May 2002, see the entire document, particularly pages 2-6; pages 64-68; and SEQ ID NO:1.	1-2, 4 and 6-19
X	KIM et al. Engineering enhancement of immune response to DNA-based vaccines in a prostate cancer model in Rhesus Macaques through the use of cytokine gene adjuvants. Clin. Cancer Res. March 2001, Vol. 7, pages 882s-889s, see the abstract.	1-2, 4, 6-7, 10, 13-14, 16-19
A	EP 0 845 530 A2 (KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO) 03 June 1998, see the entire document.	1-2, 4 and 5-19

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	"T"
"A" document defining the general state of the art which is not considered to be of particular relevance	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

01 October 2004 (01.10.2004)

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Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

Authorized officer

Quang Nguyen, Ph.D.

Telephone No.

571-272-1600

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/29640

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-2 and 4-19 with SEQ ID NO:1 and SEQ ID NO:60

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US02/29640

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-2 and 4-19, drawn to a nucleic acid vaccine of claim 1, wherein the selected antigen is PSA and methods for eliciting an immune response to a cancer associated tumor protein in a mammal using the same.

Group II, claims 1-3 and 5-19, drawn to a nucleic acid vaccine of claim 1, wherein the selected antigen is MUC-1 and methods for eliciting an immune response to a cancer associated tumor protein in a mammal using the same.

Group III, claims 1-2 and 5-19, drawn to a nucleic acid vaccine of claim 1, wherein the selected antigen is KLK2 and methods for eliciting an immune response to a cancer associated tumor protein in a mammal using the same.

Additionally, each of the aforementioned Groups I to III contains inventions which are not linked as to form a single general inventive concept under PCT Rule 13.1, depending on which SEQ ID NOS: 1-47 is used in combination with which SEQ ID NOS: 60-77. Applicants are requested to elect one of SEQ ID Nos: 1-47 and one of SEQ ID Nos: 60-77. The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking Groups I to III appear to be that they all relate to a nucleic acid vaccine comprising: (a) at least one polynucleotide encoding at least one antigenic portion of at least one amino acid sequence comprising or encoded by at least one of SEQ ID NOS: 1-47 or variants thereof (sequences encoding PSA, KLK2 and MUC-1), and (b) at least one polynucleotide encoding at least one adjuvant encoding portion of at least one amino acid sequence comprising or encoded by at least one of SEQ ID NOS: 60-77 or variants thereof (IL-18 and variants).

However, Kim et al. (Clin. Cancer Res. 7:882s-889s, March 2001) already teach a vaccine composition comprising an expression cassette encoding PSA under the control of cytomegalovirus promoter and an IL-18 gene for enhancing immune responses against prostate cancer in mouse and rhesus macaque animal models (see abstract, particularly page 885s, col. 2, lines 4-6; page 886s, col. 2, second paragraph).

Therefore, the technical feature linking the inventions of Groups I to III does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not differentiate the claimed subject matter as a whole over the prior art. Since according to Rule 13.2 PCT the presence of such a common or corresponding special technical feature is an absolute prerequisite for unity of invention to be established, and given that there does not appear to be any other technical feature common in the claimed subject matter as a whole which might be able to fulfill this role, the currently claimed subject matter lacks unity of invention according to Rule 13.1 PCT.

Consequently, the claimed subject matter was broken up into the aforementioned Groups of Inventions.

The nucleic acid vaccine of Groups I to III are different chemically and structurally one from the others, and that they require different technical consideration for attaining the desired therapeutic effects in the method of uses. For example, the nucleic acid vaccine of Group I does not require the presence of any polynucleotide encoding MUC-1 or KLK2 of the present invention. Similarly, the nucleic acid vaccine of Group II does not require the presence of any polynucleotide encoding PSA or KLK2 of the present invention. The nucleic acid vaccine of Group III does not require the presence of any polynucleotide encoding PSA or MUC-1 of the present invention. Moreover, PSA, MUC-1 and KLK2 are not structurally related, nor do they have the same biochemical activities.

Additionally, SEQ ID NOS: 1-47 lack the same or corresponding special technical feature because each SEQ ID NO. is different one from the others depending upon the presence or absence of signal sequences, introns, encoded antigens (e.g., PSA, KLK2 or MUC1), sources (human, rhesus macaque), CTL epitopes, and thus each SEQ ID NO. is different structurally and has different biochemical properties one from the others. Similarly, SEQ ID NOS: 60-77 lack the same or corresponding special technical feature because each SEQ ID NO. is different one from the others depending upon whether the IL-18 sequences contain signal sequences, introns, variants having different IL-18 activity and derived from different sources (e.g., human rhesus macaque).

INTERNATIONAL SEARCH REPORT

PCT/US02/29640

Continuation of B. FIELDS SEARCHED Item 3:

APS, DIALOG, MEDLINE, CANCER LIT., BIOSIS, EMBASE

Search terms: DNA vaccine, PSA, IL-18, prostate specific antigen, cytokine adjuvants.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes the need for transparency and accountability in financial reporting.

2. The second part of the document outlines the various methods and techniques used to collect and analyze data. It includes a detailed description of the experimental procedures and the statistical analysis performed.

3. The third part of the document presents the results of the study. It includes a series of tables and graphs that illustrate the findings of the research. The data shows a clear trend of increasing activity over time.

4. The fourth part of the document discusses the implications of the findings. It suggests that the results have significant implications for the field of study and may lead to further research in this area.

5. The fifth part of the document concludes the study. It summarizes the key findings and provides a final statement on the importance of the research.